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OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

Vol. XL MARCH-APRIL, 1948 No. 2

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PUBLISHED BIMONTHLY FOR

THE NEW YORK BOTANICAL GARDEN

AT PRINCE AND LEMON STS., LANCASTER, PA.

Entered as second-class matter April 30, 1925, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

# MYCOLOGIA

*Published by*

THE NEW YORK BOTANICAL GARDEN

IN COLLABORATION WITH THE

MYCOLOGICAL SOCIETY OF AMERICA



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## DEVELOPMENTAL STUDIES OF TWO SPECIES OF NOWAKOWSKIELLA SCHROETER: N. RAMOSA BUTLER AND N. PROFUSA KARLING *


JOHN MAURICE ROBERTS


(WITH 2 FIGURES)

The position of the genus *Nowakowskiella* Schroeter in the phylogenetic scheme of the lower fungi is undecided. It is usually placed among the cladochytriaceous fungi, a group which has been considered to contain possible transition forms between the monocentric chytrids and the filamentous phycomycetes with true mycelium. For the thallus of all the polycentric members of the Chytridiales which were then included in the Cladochytriaceae, Karling (**1932**) proposed the descriptive term "rhizomycelium." Sparrow (**1943**) divided all the chytrids into two parallel series: the Operculatae and the Inoperculatae, thus removing *Nowakowskiella* from the family Cladochytriaceae. Whiffen (**1943**) suggested that it might be better to separate *Nowakowskiella* from *Cladochytrium* on the basis of the presence of septa in the intercalary swellings, rather than on the presence of an operculum; and questioned whether the swellings in the two genera were homologous.


* A condensation of a thesis submitted to the Department of Botany and Plant Pathology, Michigan State College, in partial fulfilment of the requirements for a Ph.D. degree. Present address: Mich. Dept. of Health, Bur. of Labs., Lansing 4, Mich.



[MYCOLOGIA for January–February (**40**: 1–126) was issued February 18, 1948]

It is true of all the lower phycomycetes that the final decision as to the tenability of a phylogenetic scheme must await further studies of the cytology, development, and reproduction of all the basic forms, especially those which appear to represent transitions. At the present time only six species of chytrids have been fully investigated (Wager, **1899**, **1913**; Dangeard, **1900–1901**; Karling, **1937**; Hillegas, **1940**; and Hanson, **1945a** and **b**, and **1946**).

This study was instituted to throw more light on these possible transition forms among the lower phycomycetes.

## MATERIALS AND METHODS

*Cultivation.* The original collection of *N. ramosa* used in this investigation was found by Professor E. A. Bessey on grass leaves in distilled water to which had been added debris from a rain-water cistern. This species and *N. profusa* were subsequently collected in a garden pool and from the relatively still water along the edge of a river.

Cultures for study and for stock maintenance were grown on hemp achenes and cellophane in sterilized river water in petri dishes. For the study of living specimens at high magnification hanging drops were suspended from cover slips on rubber washers sealed to the slides with either petroleum jelly or waterproof cement.

It was found that sterilized river water containing cellophane as the only other source of food supported abundant development and reproduction of *N. ramosa*, whereas distilled water, physiological saline, and Knop's solution would not sustain growth of this species for more than one transfer.

*N. ramosa* would not parasitize seedlings of barley, corn, oats, rice, rye or wheat; nor would this species attack the contents of cereal grains or hemp achenes although it would grow well on the pericarps alone. Cotton fibers and cellophane chips were digested directly by the fungus, but chemically prepared cellulose acetate was not attacked.

On initial isolation, *N. ramosa* required a temperature between 16° and 18° C. for cultivation and had to be gradually acclimated to temperatures above 20° C. *N. profusa* grew readily at 20° to 28° C. from the beginning and was inhibited by the lower temperatures.

*N. ramosa* indicated an affinity for free oxygen, in that this species produced better growth and zoosporangial development at the surface of the liquid in petri dishes and could not be recovered in the living state from cultures in test-tubes. *N. profusa,* on the other hand, produced more zoosporangia appressed to the bottoms of the petri dishes and developed freely under one to two inches of water in Ehrlenmeyer flasks.

*Staining.* Most of the cytological data in this paper are taken from material which had been killed and fixed in Sass' modified Bouin's fluid, Belling's Navashin's fluid, Flemming's weak fluid—undiluted and one-half strength, Carnoy's fluid, and the Zirkle-Erliki fluid for mitochondria. For cytological details Tuan's smear modification of the haematoxylin method and Flemming's triple stain gave the best results. Material stained by the haematoxylin method was whole-mounted directly from the last water-rinse into glycerine jelly, thus alleviating the difficulty of matching cytological structures which is encountered in the use of serial sections. With extreme care, material stained by Flemming's method could be dehydrated by running large volumes of the solutions over the specimens on a large glass plate and then mounted in balsam.

As an aid in the observation of living specimens, a drop of one per cent aqueous Porrier's blue was allowed to diffuse into the mount from the edge of the cover slip.

Zoospores were stained by the Feulgen reaction, Gram's stain and the crystal violet method described by Cotner (**1930**).

*Microchemical Tests.* Thallus walls of both species give a positive reaction to the chitosan test as described by Johannsen (**1941**). Picric acid gives, as Nabel (**1939**) described, a yellow coloration that cannot be washed out. When 75 per cent sulfuric acid is added to material treated in this way, the entire thallus dissolves. The sporangium and apophysis walls of both these species also react slightly to chlor-iodide of zinc, indicating the presence of some cellulose. Both the chitin and the cellulose seemed to be present in the same layer in both species. An inner layer in which neither cellulose nor chitin was demonstrated to be present, appeared in the sporangial wall of *N. ramosa,* but not in *N. profusa.* There was no evidence of an evanescent inner wall of chitin such as Nabel (**1939**) found in *Rhizidiomyces bivellatus.* These spe-

cies of *Nowakowskiella* do agree with *Rh. bivellatus* in that the walls of the filaments and rhizoids respond to tests for chitin and not for cellulose. Hillegas (**1940**) found the walls of the zoosporangia of *Endochytrium operculatum* to give a weak cellulose reaction, as is true with the two species being considered here, but does not mention their behavior with tests for chitin.

In the case of both *N. ramosa* and *N. profusa*, treatment with ruthenium red stains none of the fungus except the operculum. The reddish violet color acquired by this structure indicates that it is primarily of a pectic nature.

The "oil" globules and refractive matter reacted alike, whether in the zoospores, filaments, spindle-organs, or rhizoids. With Sudan IV they stained golden yellow, became light brown after standing in osmic acid, and were dissolved by acetone. These findings do not add any more information as to the composition of the "oil" in the chytrids than Karling (**1937**) and Hillegas (**1940**) obtained with *Cladochytrium replicatum* and *Endochytrium operculatum*, respectively; that is, they seem to be of a fatty nature but may be more complex. Treatment with saturated aqueous picric acid or with dilute aqueous eosin did not show any indications of proteinaceous matter with the fatty globules. This possibly was due to the immiscibility of the water-dissolved and the fatty matter. With picric acid the nuclear caps and cytoplasmic strands stain a bright greenish yellow.

## DEVELOPMENT OF N. RAMOSA

*Structure of the Zoospore.* The typically single refractive globules seen in the living zoospore of *N. ramosa* are anterior or lateral, never posterior, to the centrally-located nucleus, a condition similar to that in *Solutoparies Pythii* (Whiffen, **1942**). Atypical spores may exist containing two or three globules, as described for *N. elongata* by Karling (**1944a**) and for *Sporophlyctis rostrata* by San-Chiun Sen (**1944**). Variability as to the refractive globule in the genus *Nowakowskiella* is common. Among the other species, *N. elegans*, *N. hemisphaerospora*, and *N. delica* (Matthews, **1928**; Shanor, **1942a**; and Whiffen, **1943**) have been described as having a single spherical globule; *N. elongata* (Karling, **1944a**) usually has a single globule but may have two. The refractive ma-

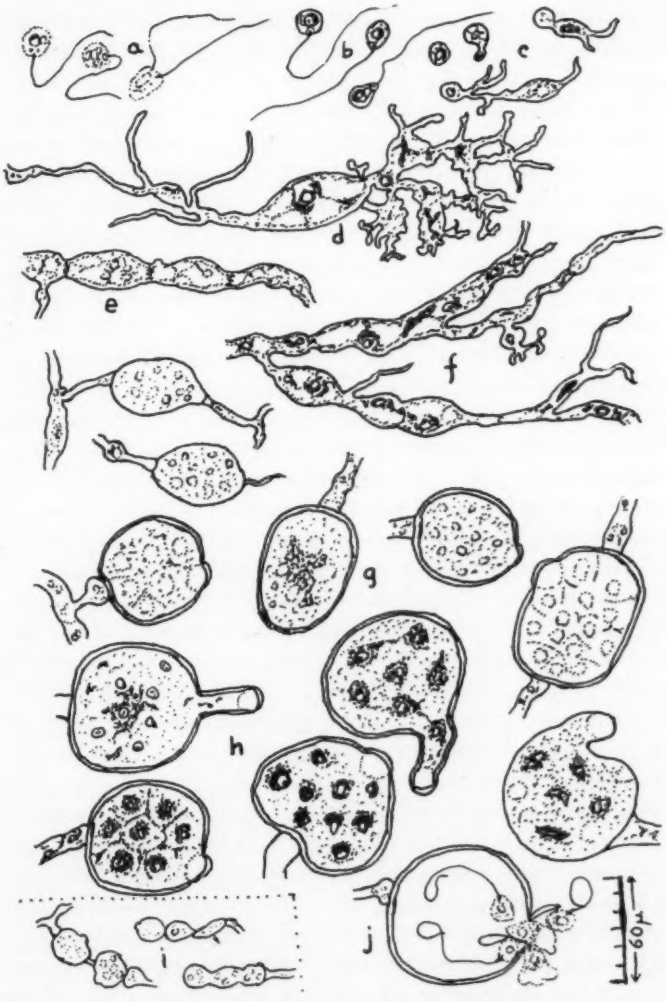


FIG. 1. *Nowakowskiella ramosa.*

terial in *N. macrospora* (Karling, **1945a**) is composed of a disc-shaped globule and numerous minute granules; and *N. granulata* (Karling, **1944a**) is characterized by the presence of numerous golden brown granules.

Also visible in the living spore is a minute granule, the blepharoplast, in a small vacuole posterior to the nucleus. From this granule the single flagellum extends into the surrounding medium. In the abnormal bi-, tri-, or quadriflagellate spores, which occur in *N. ramosa*, there is a blepharoplast for each flagellum. These may be located in the same or in different vacuoles. Although the multiflagellate spores may have more than one refractive globule, they contain only one nucleus. The biflagellate-uninucleate condition of *Nowakowskiella* sp. spores has also been reported by Ellison (**1945**). The biflagellate spores are usually somewhat larger than the uniflagellate (10.6–21.3 $\mu$ as compared to 4.8–9.7 $\mu$), and are seldom the same size. Since fusion of two spores has never been observed and since tri- and quadriflagellate forms are found, it is believed that these multiflagellate spores are not evidence of sexuality in this species. The author prefers to believe for the present that this condition may be due to improper cleavage as reported by Cotner (**1930**) for *Blastocladia* and by other workers for other chytrids. Evidence in favor of the uneven cleavage theory is given by the presence of nonflagellate spores consisting of little more than a nucleus in the same slides as the multiflagellate spores. Further studies may throw more light on this strange attachment of the flagellum of one spore to the nucleus of another.

Whatever the reason for the alignment of more than one flagellum with a single nucleus, it appears to be evidence that at least the initials of these appendages are laid down between the times of division of the sporangial plasma into the spore initials by furrowing and the final separation of the mature spores preceding their escape. The flagella are evident immediately on those spores left in the zoosporangium after the first mass has escaped. Their presence was also observed by Karling (**1937**), Hillegas (**1940**), and Couch (**1945**) and is considered to support the belief that the flagellum is formed while the spore is still in the sporangium. This time of formation is also indicated in that spores attempting

to separate from the mass at the orifice may be observed held by a fully formed flagellum caught in the exit papilla by another spore. Some of these caught flagella have loops or vesicles at the ends. When the spore has freed itself, the loop is no longer evident. Hillegas (**1940**) described similar loops on the flagella of immature spores of *Endochytrium operculatum;* Berdan (**1941b**) found loops in various positions on the flagella of *Catenochytridium carolinianum* zoospores; and Ajello (**1942**) reported them on the flagella of occasional zoospores of *Polychytrium.* These looped flagella are probably the results of developmental irregularities and not homologous to the knobbed modification of the whip-lash flagella found by Ellison (**1945**) in some of the Mycetozoa. Karling (**1945b**) and Hanson (**1945a**) have recently described loop formation in the absorption of the flagella when the spores become sessile.

Usually there is a second vacuole, other than that containing the blepharoplast in the spores of *N. ramosa.* Its position is lateral or anterior, frequently opposite the globule in relation to the nucleus. Under unfavorable conditions, such as too long exposure to distilled water, this vacuole may swell until the spore has become distended into little more than a tonoplast about a huge (in relation to the usual spore size) vacuole. When the spore is in this distended condition the nucleus and nuclear cap are compressed into the posterior end near the point of insertion of the flagellum and motility by flagellar or amoeboid action ceases.

As is becoming increasingly evident among the chytrids, the most conspicuous structure in a stained zoospore of *N. ramosa* is a crescent-shaped nuclear cap, surrounding a third or more of the centrally-located nucleus. The finding of nuclear caps in the zoospores of the chytrids prompted Ajello (**1942**) to suggest that these extra-nuclear structures are not as important phylogenetically as once thought. In the spores of this species the nuclear caps may vary from more or less thin crescents to cups or spheres. The crescent-form and the cup-form are usually tilted so that the cap is oriented with the thicker part to one side as may occur in the spores of *Catenochytridium laterale* (Hanson, **1946**) and not directly anterior to the center. The spherical form appears to be a hollow ball which is filled with the nucleus.

Lateral to the nucleus is a large clear space, which appears to be left by the dissolution of the refractive globule of the living spore. If this space does represent the position of the globule, it indicates a fixed position for that structure relative to the nucleus and nuclear cap. For *Endochytrium operculatum,* Hillegas (**1940**) considers a similar clear space to be the nucleus.

Most of the fixed and stained spores exhibit a distinct blepharoplast and rhizoplast associated with the flagellum, such as have been described in chytrid zoospores before (Berdan, **1941**; Karling, **1937**; and Hillegas, **1940**). Ellison (**1945**) uses the presence of these structures for designating that the swimming organelle of the phycomycete zoospore is a true flagellum. Usually the stained spore lies so that the thin rhizoplast can be seen passing diagonally from the blepharoplast to a point of attachment at the nucleus in the vicinity of one of the pointed horns of the cap. Karling (**1942**) described the nucleus tapering to the point of attachment of the flagelum in the zoospore of *Septochytrium macrosporum,* in a similar manner to the sub-triangular nuclei in the zoospores of *Blastocladiella simplex* and *Blastocladia* (Matthews, **1937**; Cotner, **1930**). Either a similar tapering structure or two granular cytoplasmic strands (one or both functioning as a rhizoplast) extend from an apex at the blepharoplast to the posterior side of the tilted nuclear cap in some spores of the present species. Only one of the strands joins the central structures at the tip of a horn of the cap. If this really be a conical extension of the nucleus, a second opening in the cap must be presupposed; if it be strands, the structure is like that found in *Monoblepharella Taylorii* (Springer, **1945**) and occasionally in *Blastocladia* (Cotner, **1930**).

When the spore becomes sessile (between four and twelve hours after release from the sporangium) the well-defined nuclear cap disappears, in contrast to the occurrence in *Cladochytrium replicatum, Endochytrium operculatum,* and *Polychytrium stromaphilum* in which the cap may persist during the early stages of germination (Karling, **1937**; Hillegas, **1940**; Ajello, **1942**). In place of the cap there is a deposit of large granules around the outside of the nuclear membrane. Larger granules also become apparent in the cytoplasm, radiating in elongated masses from the nuclear to the plasma membrane, lining the inside periphery of the cell, and sur-

rounding small vacuoles. Examination of the spores which appear to be in transitional stages in the disappearance of the cap indicates that this structure is the source of the majority of these large granules. The clear space which is adjacent to the nucleus and the nuclear cap in the swimming spore may or may not be present or may be partially filled with granules. Here again the behavior of this clear space in the stained spore parallels that of the globule which becomes smaller and disappears in the living. The nucleus contains a central nucleolus and a fine reticulum.

*Germination of the Spore.* Between six and ten hours after the zoospore of *N. ramosa* has become sessile, it puts forth a single germ-tube which usually sends off one branch before it has grown longer than the diameter of the spore. Although the germ-tube may branch two or three times before there are any swellings formed, the most common development results in a primary swelling, which may be homologous to the primary spindle-organ in *Cladochytrium replicatum* (Karling, **1937**), before the second dichotomy occurs. A very young thallus with one branch and one swelling resembles the early stages of the type 3 formation of the apophysis in *Catenochytridium laterale* (Hanson, **1946**). The nucleus may remain in the spore case until the primary swelling has formed or may migrate into the unswollen tube which enlarges around it, as Hillegas (**1940**) has described for *Endochytrium operculatum*. If the nucleus remains in the spore case, it may divide and the daughter nuclei migrate to the primary swelling, one at a time, thus differing in this respect from *Cladochytrium replicatum, Endochytrium operculatum,* and *N. hemisphaerospora* (Karling, **1937**; Hillegas, **1940**; Shanor, **1942a**). In order to pass through the narrow filament from the spore-case to the enlargement, the nucleus becomes elongated and narrow, in the same manner as Karling (**1937**) reported for *Cladochytrium replicatum.*

After the primary swelling has formed there is no further growth originating from the spore, even though the spore case may persist throughout the life of the thallus. The persistence of the spore case may be ontogenetic evidence of rhizidiaceous ancestry. Unlike *Cladochytrium hyalinum* (Berdan, **1941a**) the spore case is not emptied of cytoplasm before the formation of the primary swelling whether the nucleus has migrated into the tube or not.

The branches of the germ-tube may be so close together that there appear to be two or three germ-tubes from a single spore under low magnification. However, this is a false impression; and the fact that a single germ-tube is formed seems to be important in the light of Couch's (**1945**) having used the two germ-tubes in *Catenaria* as one of the points for transferring that genus from the *Chytridiales* to the *Blastocladiales*.

*The Vegetative Portion of the Thallus.* After the primary swelling and its rhizoidal offshoots have become established, the thallus continues to grow, branching dichotomously and producing additional nucleate swellings. No cross walls are formed in the extension of the thallus. It differs in this respect from *Cladochytrium replicatum* and *C. tenue, Catenomyces,* and *Catenaria*. For one or two days in *N. ramosa* most of the growth consists of the establishment of spherical, fusiform, and irregular swellings appressed to and imbedded in the substratum. In the mature thallus numerous much-branched rhizoids arise from the thallus and from the isthmuses between. When the fungus is grown in grass leaves, the swellings may occupy part or all of the cavity of the host cell; and the rhizoids dissolve their way into and ramify in the cell walls. On cellophane chips, the swellings are appressed to the surface; and the rhizoids lie in channels of fluid digested into the solid substratum. The rhizoids are short and may be expanded in spots, especially at the points of much-branching, into bladder-like formations with thin walls and large vacuoles. Another polycentric operculate chytrid forming thick, short rhizoids in cellophane is *Catenomyces persicinus* (Hanson, **1945a**). Some of the bladders are so great in diameter in places of pronounced branching that they can be distinguished from the centers of development only by the lack of nuclei, and some by the thinner wall. Except for *Nowakowskiella* not containing cross-walls this network of rhizoids and swellings resembles the thallus of *Megachytrium Westonii* (Sparrow, **1933**) more closely than it does any of the other polycentric chytrids.

The most pronounced inclusions in the living protoplasm of the vegetative system are the refractive globules, which resemble the globules of the zoospores, except for the former's great variation in size. The presence of similar refractive globules in the intra-

matrical portion of the thallus has been reported before for *N. profusa* (Karling, **1944**), as well as for *Cladochytrium replicatum* (Karling, **1937**), *Catenomyces persicinus* (Hanson, **1945a**), *Cladochytrium hyalinum*, and *Catenochytridium carolinianum* (Berdan, **1941a** and **b**). Few scattered minute granules are present in the rhizoids which have digested their way into the substratum; but wherever a swelling occurs, there is an accumulation of globules, which may fill the swelling as a single mass in a mature thallus.

The hyaline cytoplasm appears to fill the absorptive rhizoids and to surround and divide diagonally large vacuoles in the swellings. The vacuolate nature of the cytoplasm of the vegetative portions of *Cladochytrium replicatum*, *Endochytrium operculatum*, and *Catenaria* has been described by Karling (**1937**), Hillegas (**1940**), and Couch (**1945**), respectively. The globules are in intravacuolar groups, held in the cytoplasmic strands.

Fixing and staining procedures dissolve out the refractive matter and accentuate the structure of the cytoplasm and the nuclei. If osmic acid is used in a fixative and is not bleached out, the place occupied by the refractive matter contains a brownish, amorphous mass. Otherwise, the spaces occupied by the globules are clear except for loose clumps of densely-staining granules. Similar granules are found scattered through the cytoplasmic strands. The number of granules present in *N. ramosa* and *N. profusa* seems to be greater than that for *Cladochytrium replicatum* (Karling, **1937**). In many instances the nuclei are surrounded by masses of these granules. In their affinity for the nucleus these granules resemble those which make up the nuclear cap of the zoospore.

In older thalli strictures in the isthmuses between the swellings may be filled with cytoplasm, often of a denser appearance than that in the swellings, forming a type of pseudoseptum. Similar bands of material were found in the rhizoids of *Septochytrium variabile* by Berdan (**1942**).

### THE REPRODUCTIVE PORTION OF THE THALLUS

*Flexuous Filaments.* Arising from the sides or ends of the vegetative swellings or from the isthmuses between are long,

flexuous, usually extramatrical filaments which branch by repeated dichotomies. These filaments may be isodiametric throughout their entire length or may vary considerably in diameter. Elongate fusiform swellings, which are smooth in outline, may usually be found regularly in the proximal one-third of each filament. Rarely were secondarily developed, typical absorptive-vegetative centers found arising from these swellings. Distal to this area, the swellings are irregular in shape and length: being fusiform, spherical, or even triangular at points of branching, to mere undulations in the otherwise parallel filament walls. The narrower portions range in diameter from 1.5 to 2.5 $\mu$ in the more distal segments, while the swellings in these segments are only 2.0 to 4.0 $\mu$. The length of the narrower portions is 4.0 to 40.0 $\mu$, as opposed to 11.5 to 30.0 $\mu$ for the swellings. In this area it is difficult to draw a clear-cut differentiation as to what constitutes a swelling and what an isthmus. If one overlooks the small differences in diameter, it is possible to consider segments as long as 300 $\mu$ as lacking true swellings. The swellings and isthmuses in the proximal portion are more easily differentiated, since their sizes are 2.5–5.0 $\mu \times$ 6.0–11.0 $\mu$ and 1.5–2.5 $\mu \times$ 4.0–30.0 $\mu$, respectively. It is not uncommon to find one of these flexuous filaments almost twice as wide in the distal portion as in the proximal. Lateral branches may form at almost right angles and remain only 1.0 to 1.5 $\mu$ in diameter.

Internally these filaments may resemble true tubular coenocytes. The cytoplasm in the younger thalli contains many deeply-staining granules and small refractive globules, and is netted with small vacuoles. In older thalli the vacuoles are longer, surrounded and crossed by thin strands of minutely-granular cytoplasm. As in the vegetative system, pseudo-septa of thick cytoplasm may be found in points of constriction.

The presence of nuclei in these filaments is variable. They are usually absent in the narrow lateral branches, and are usually present in the swellings. Up to six nuclei have been found in a single enlargement. Mature thalli may bear irregularly swollen filaments or filaments lacking swellings in which rounded nuclei (2.0 $\mu$ in diameter) are scattered throughout their lengths. As many as fourteen nuclei have been observed evenly distributed in a fila-

ment of *N. ramosa* 485 $\mu$ long from its origin to the cross-wall separating the zoosporangium from the rest of the tube. The regular distribution and the rounded shapes and lateral position of the nucleoli in the nuclei make it appear that these structures are fixed in their places, rather than passing through the filaments. Karling (**1937**) described the moving nucleus in *C. replicatum* as elongate and densely-staining. Hillegas (**1940**) reported that the nuclei of *Endochytrium operculatum* elongate only when passing through a constriction in a rhizoid. Since the author has observed nuclei scattered in the majority of the filaments which were alive just prior to the time of killing and fixing, it is his opinion that these structures are typically nucleate in both *N. ramosa* and *N. profusa.*

*Formation of the Zoosporangium.* Each of the flexous filaments described above may bear zoosporangia which are terminal, intercalary, or on short lateral branches. Most of the zoosporangia are extramatrical, rarely intramatrical. The author has never observed a typical vegetative swelling producing zoospores. In this way these species of *Nowakowskiella* differ from *Cladochytrium replicatum* (Karling, **1937**) and *Megachytrium Westonii* (Sparrow, **1933**). The thallus of *Physocladia* (Sparrow, **1932**) exhibits a similar specialization. The differentiation of the thalli into vegetative and reproductive portions is pronounced in *N. ramosa* and *N. profusa.*

On actively-growing thalli of *N. ramosa,* the continuation of the filament distal to the zoosporangium is usually a fine thread which shrivels as the sporangium matures. In the few instances where this thread has persisted, it has been found to be anastomosed with another, larger flexuous filament. Most often a filament of *N. ramosa* branches near its end, and each of the equally long branches is terminated by a zoosporangium. Examination of thalli bearing predominantly zoosporangia gives the impression that these reproductive bodies are terminal in *N. ramosa.* The filament below the sporangium of *N. ramosa* is swollen into an infundibiliform sub-sporangial swelling.

Elongate exit tubes develop on those sporangia that are intramatrical or surrounded by a zoogleal slime, and do not extend beyond the outer edge of the enveloping material. A zoosporangium

of *N. ramosa* may bear one to three branched exit tubes or papillae, only one of which is functional.

At their inception the swellings which develop into zoosporangia are usually intercalary. If the enlargement begins near the tip of the filament, the tip may be included in the rounding-out of the sporangial rudiment; or the tip may shrivel, as described above. The swellings are usually fusiform-elongate at first, resembling those in the lower third of the flexous filaments. They increase in circumference more rapidly than they do in length until they have become spherical in shape. Deviations from this typical spherical shape are pyriform, ovate, obtusely-branched, obtusely-triangular, and hourglass-shaped. When the rudiment is about one-half its mature size a cross-wall forms, dividing the swelling into two unequal parts. This time of delimiting the incipient zoosporangium differs from that of *N. delica* in which the cross-septum is formed when the swelling has reached mature size (Whiffen, **1943**). The smaller portion of the swelling continues to enlarge into an evenly-shaped funnel form in *N. ramosa* so there is no constriction at the cross wall.

When the incipient sporangium has reached mature size, the wall and cross-septum increase in thickness. At one point, lateral, subapical, sub-basal, or rarely apical, the sporangium wall forms a disc-shaped area which is thicker and more refractile than the rest. Around the circumference of this disc, the wall is very thin, seeming to consist of little more than the external layer of the immature thin-walled swelling. If exit tubes or papillae have formed, this disc is at the tip of a tube or a papilla; otherwise it is flush with the surface of the wall. This disc becomes the pectinaceous operculum which bulges as a slight convexity on the mature sporangium wall.

*Zoosporogenesis. Appearance of Living Material.* The behavior of the protoplasm in developing sporangia of *N. ramosa* is essentially the same as that described for other chytrids (Berdan, **1941a** and **b** and **1942**; Couch, **1945**; Hanson, **1945a** and **b**; Hillegas, **1940**; Karling, **1937**, **1944a** and **b**, and **1945a**, **b**, and **c**; Whiffen, **1942** and **1943**). Because of this similarity, the author will refer to the observations of other workers only where differences in structure and behavior of the organisms seem significant.

As the swelling which develops into a zoosporangium grows, the refractive content of the otherwise hyaline cytoplasm begins to increase in the enlargement. At the time of cross-wall formation the refractive matter appears to be concentrated in the center of the swelling. At this time several large vacuoles may be observed in the incipient sporangium, and a few large globules are present. Unlike the condition in the monocentric chytrids, the filaments supporting the sporangia of this species are not devoid of cytoplasm when cross-walls are formed. Shortly after the formation of the cross-wall the large globules in the growing sporangium increase in size and number. Later these globules again disperse, and the evenly granular appearance returns to the cytoplasm. During this stage of evenly granular appearance, the sporangium attains its mature size. Also during most of the granular stage the vacuoles are not as evident as before. Soon, however, elongate, narrow vacuoles can be seen forming in the cytoplasm, dividing it into irregularly-shaped masses. This cleavage progresses primarily from the outer edge toward the center; but additional vacuoles may be found arising independently of the primary furrows, cutting the cytoplasm from the inside out, radially, or tangentially. In each of the masses so formed the minute oil droplets coalesce into a single, or at the most two, globules. Except for the refractive globules, the cytoplasm acquires a homogeneous appearance and swells until the vacuolar divisions are obscured. In this stage the cytoplasm completely fills the sporangial swelling, except for a clear space which has formed below the operculum. Because of its spherical shape, maintained at the expense of the rest of the contents, this space appears to be filled with cytoplasm such as in the body of the sporangium up to the clear space which is just below the operculum at the tip of the tube.

*Appearance of Stained Material.* In the stained material the development of the sporangium has been followed by the study of cells of different sizes, using the size as an indication of relative maturity. This method seemed practical because the size of the swelling and the number of nuclei ordinarily paralleled each other.

When the small intercalary swelling first appears it is filled with a granular cytoplasm and exhibits one nucleus about 2 $\mu$ in diameter. The deeply-staining granules are small, with larger granules

massing in streaks through the cytoplasm. Often these streaks are associated at one end with the nucleus, an observation similar to that of Karling (**1937**) in the newly nucleated spindle-organ of *C. replicatum*. The author's observations on *N. ramosa* do not affect the theory that the strand represents the line of passage of the nucleus. As the swelling enlarges these strands disappear and the granules become evenly distributed throughout the sporangium rudiment. The tapering portion of the enlargement which is to become the subsporangial swelling is filled with cytoplasm and may contain one or more nuclei like those found in the sporangium rudiment. Several large nuclei are found in the rudiment before it has enlarged very much. In a few preparations the first nucleus in the rudiment has been observed to be dividing; but there was no proof discovered that all the nuclei in it came from continued divisions of this single nucleus or that some of them might not have migrated into the swelling from the proximal or distal filaments. The solution of this question is further confused since there are several nuclei in the swelling before the septum cuts it off from the rest of the filament.

As the swelling continues to enlarge into a spherical form and more nuclei are found in it, the deeply-staining granules aggregate loosely in the center, leaving the remainder of the cytoplasm less densely granular. When the rounded form of the swelling is established, the adjacent portion of the proximal filament has swollen to be infundibuliform. The distal continuation of the filament begins to shrivel back to the rounded swelling in those sporangia which appear terminal at maturity although intercalary in origin. If the distal filament persists, it may be swollen or not. The flexuous filaments on which the sporangia are borne are usually almost devoid of nuclei and highly vacuolate by the time the sporangium is mature. The vacuolate nature of the cytoplasm is less prominent nearer the sporangium, and nuclei may be present in the swollen filament immediately below the sporangium. This is also true of the distal continuation of the filament if the sporangium is truly intercalary, or if this portion has anastomosed with another filament as described above.

The granular mass in the center of the sporangium disintegrates as a densely granular reticulum spreads throughout the

swelling. The nuclei become aligned on strands of this reticulum in such a way that observation of subsequent nuclear divisions is made difficult. Some strands of reticulum extend down to where the tapering of the swelling begins; and at this place, a thick plate of granules extends across the swelling. As this plate becomes more apparent, the outer wall of the sporangium begins to thicken, and the papillar swelling begins to grow through any material present that will inhibit the free escape of the zoospores. As the plate which will form the cross-septum becomes more compact and densely granular, it bulges up from the filament into the sporangium, as though the pressure exerted in the filament were greater than that in the sporangium itself. The granules forming the reticulum again become dispersed, and the cytoplasm is again uniformly granular. The granules present at this time are minute and deeply-staining. The area immediately below the exit papilla is devoid of stainable cytoplasm at this stage.

The peripheral wall of the sporangium becomes thick and deeply-staining, and the arched operculum is evident as a lightly-stained dome which is thick in the center and tapers off toward the edges. At the point where the operculum is attached to the rest of the wall, the wall is not thickened but has remained a thin ring. By the time the operculum is fully developed the cross-septum between the sporangium and the filament has the same structure as the rest of the sporangium wall.

After the walls are completely formed, the cytoplasm begins to be furrowed at the periphery by the elongated vacuoles mentioned above. This furrowing by vacuoles progresses centripetally and laterally until each nucleus has a mass of cytoplasm cut out around it. The rest of the cytoplasm in each of these areas becomes clearer as the deeply-staining granular material collects arouhd each of the nuclei. The granular mass around the nucleus consolidates and takes the form of a loosely-packed, over-sized nuclear cap. After the cytoplasm swells so as to obscure the divisions and fills the sporangium case, the nuclear caps become more compact and more evenly and deeply-staining. The nucleolus must function independently of the nuclear cap, because it is evident as a small, dark body in the center of the nucleus before and after the granules begin to clump about the nucleus.

The above observations as to the formation of the nuclear cap tend to support Karling's (**1937**) belief that this structure arises from chromatic bodies or granules in the cytoplasm and is external to the nucleus itself. As with Karling's (**1937**) and Hillegas's (**1940**) material, *N. ramosa* demonstrates easily-stained nuclear caps after non-chondriosomal fixatives. It is of interest to note the similarity of behavior between the granules which form the nuclear cap and the refractive globules in their aggregation and dispersion in the incipient sporangium. The presence of similar deeply-staining granules in the vegetative portion of the thallus which in the living state contains a great deal of "oil globule" material indicates that the dispersed cap material and the "oil" may be closely associated throughout the life of the thallus. It may be that the granular material is carried dispersed in a lipoidal medium; and extrudes the lipid as the "oil globule" of the zoospore in the process of aggregating as the compact nuclear cap. The proximity of the cap and the globule in the swimming spore appears to support this belief. Since both the globule and the cap lose their distinctive identities before and during the germination of the spore, the suggestions by Karling and Hillegas that the cap is food-material which is used up in the process seem to apply to *N. ramosa*.

Although the present author has not attained the perfection of staining the dividing nuclei demonstrated by the investigators at Columbia University (Karling, Hillegas, and Hanson), he was able to observe definite mitotic figures in both species of *Nowakowskiella* studied. The nuclear behavior differs in these species from that in *C. replicatum, Endochytrium operculatum* and *Catenochytridium lateral*e (Karling, **1937**; Hillegas, **1940**; Hanson, **1946**) in that the nucleus does not increase appreciably in size before division and divides repeatedly during the enlargement of the incipient sporangium before and after the formation of the cross-wall. Although the division of all the nuclei in a single sporangium occurs simultaneously, it is not absolutely synchronous, since nuclei in various stages of division may be observed in a single swelling.

*Zoospore Escape.* For an indefinite time after maturation of the zoosporangium and zoosporogenesis, no further visible change

takes place in the sporangium. When conditions are naturally or artificially conducive to zoospore discharge, the entire contents begin to revolve in an undivided mass within the zoosporangium wall. While the cytoplasm is in motion, the operculum is forced clear off or is thrown back as on a hinge, and the subopercular material is expelled. After the operculum dehisces, the cleavage lines in the sporangium again become distinct and the irregularly shaped individual spores are evident.

The zoospores escape as individuals. The first to pass through the orifice flow through rapidly, apparently forced from behind by those adjacent to them. A mass of zoospores may form at the outside of the exit papilla, but the individuality of these spores is evident both before and after their escape. The present author would like to suggest that the spores escape into a mass, rather than *en masse*. In both species of *Nowakowskiella* considered in this paper, a single spore must be elongated and distorted to pass through the exit orifice; since the opening is but one-half the diameter of the typical spherical zoospore and the surrounding sporangium wall is rigid compared to the naked protoplast of the spore. No vesicle or any other confining material was found enclosing the mass of spores at the tip of the papilla. Whenever the outline of such a structure was suspected, continued observation showed the line to be the entangled flagellum of one of the spores in the mass. The existence of the mass is short-lived, only long enough for the spores to round up, free their flagella, and swim away.

The number of zoospores formed per sporangium varies. The zoosporangia producing the fewest spores have been observed in cultures of *N. ramosa* when very small sporangia on short filaments were observed early in the life of the thallus and contained only four or eight spores. The average number of spores for a normal zoosporangium in this species has been determined as about thirty-six.

*Fusion of Cells and the Resting Bodies of N. ramosa.* In many of the older cultures resting bodies were formed developing from a pseudoparenchyma as described by Butler (**1907**). These cells have yellowish walls of considerable thickness, which are smooth in most cases. However, it is not uncommon to find resting

bodies with the outer wall corrugated and with the inner wall exhibiting striations.

When the culture or the strain in culture has aged considerably (two or three months, for transfers were ordinarily made every two or three weeks), instead of being so fruitful in producing zoosporangia, short swollen lateral outgrowths form from the flexuous filaments.

These outgrowths may be merely irregularly-shaped enlargements which are formed by the filament swelling on one side only, or they may be short side branches consisting of thin isthmuses terminated by club-shaped tips. Two of these swellings come into contact and become superimposed upon each other. The author had difficulty in determining whether these cells actually fuse or if their cell walls merely adhered to each other. Whichever occurs, they are found in this appressed condition often enough to indicate that they are fastened together in some manner. Both cells retain their rounded shape, being flattened only where they are in contact. The cytoplasm in these cells is minutely granular, and each of them contains a single nucleus. In some cases the resultant structures are pretty definitely single cells with two rounded hemispheres, produced by the fusion of the two swellings. Whatever the case may be, nuclear fusion has not been observed. Karling (**1944a**) has described the formation of pseudoparenchyma from single swellings and single lateral branches, as well as from the fused tips of branches in strains of *N. ramosa* collected in Brazil. This "pooling" of the contents of two cells from different filaments may be homologous with the behavior of the two cell-potentials from which arise the resting spores of *N. hemisphaerospora* (Shanor, **1942a**). In the Brazilian strains in which the pseudoparenchyma is produced without "fusion" of cells, but from a single cell, it is possible that a procedure such as has been reported for *N. hemisphaerospora* occurs during the formation of the pseudoparenchyma. The first change that occurs in the "fused" cells is the formation of large vacuoles with the tonoplasts extending across the cells so as to separate them into four to eight clear areas. A cell wall is laid down where each line of cytoplasm is present, in such a way that each of the "fused" cells is divided into as many daughter cells as there were vacuoles. Not all of these daughter

cells contain nuclei. Those that do not are either continuous with the mother filament or give rise to apophyses as described below.

The nucleate cells in the psuedoparenchyma may produce short off-shoots which have clavate tips. These tips are filled with a granular cytoplasm as they enlarge. After they have attained a more or less spherical shape, the nucleus from the pseudoparenchyma cell migrates into the apical swelling, and a plate of cytoplasm forms across the base of the rounded resting-cell initial. The nucleus takes a central position in the cell surrounded by a loosely-packed mass of granules. From this center, strands of granules radiate to the periphery of the cell. The presence of similar chromatic granules has been reported for *Polyphagus Euglenae, C. replicatum,* and *Endochytrium operculatum* (Wager, **1913**; Karling, **1937**; Hillegas, **1940**). The nucleus in this resting cell was observed not to divide but to remain single and centrally placed. The uninucleate condition until mature size is attained has also been observed in *C. replicatum* and *Endochytrium operculatum* (Karling, **1937** and Hillegas, **1940**).

The living cell shows a dispersion of oil droplets evenly throughout the cell and then a re-coalescing of the droplets into about eight large globules. Stained material shows the deeply-staining granules to follow the same procedure. The cytoplasm remains reticulate. Although the peripheral wall is thickened slightly by the time the cross-septum is completed, after the cell has become more stabilized as to its internal structure a striated and thicker inner wall is laid down interior to the original.

These resting bodies have not been observed to germinate in the present strain, but Karling (**1944a**) observed them to produce zoospores directly or to function as prosporangia.

### DEVELOPMENT OF N. PROFUSA

*N. profusa* differs from the other species of *Nowakowskiella* in having a coarse, profuse rhizomycelium with few or no well-defined spindle-organs, smaller spore size, and yellow-brown resting spores (Karling, **1941**). The present strain fits Karling's description with the exception that no resting spores were observed.

In most instances the development of this species is the same as has been described for *N. ramosa* in the preceding section. The

first differences between the two species to be obvious are that the flexuous filaments of *N. profusa* are almost isodiametric for their full length and that the asexual cycle of *N. profusa* takes longer for completion than does that of *N. ramosa*. Other less obvious differences are noted below.

The refractive globule is usually lateral and posterior to the nucleus; and smaller refractive globules in the posterior area may be present as Karling (**1945a**) described for *N. macrospora*. Unlike the globule in *N. ramosa* (Butler, **1907**) and in *Endochytrium operculatum* (Hillegas, **1940**), that in *N. profusa* is not fluid nor does it change shape. When the spore is escaping from the zoosporangium or is resorting to amoeboid motion, as it may do repeatedly, the globule remains in its posterior position and keeps its spherical shape. Under such circumstances, hyaline pseudopodia extend anteriorly and laterally from the spore, while the granular and refractive material of the spore remains faintly delimited as an internal spherical mass.

The vacuole, other than that containing the blepharoplast, most often is about the same size as the refractive globule or slightly smaller. The deeply-staining portion of the nuclear cap may be less dense than that in *N. ramosa*. Frequently an enclosed clear sphere containing a nucleolus-like structure, all surrounded by cap, is exhibited in such cases. This is thought to be further evidence that the nucleus is centrally located within the cap.

When the zoospores are escaping, the flagella have been observed to be entangled so frequently that the present author feels that this might be the mechanism explaining the massing of spores on discharge. The average number of spores in a typical sporangium is between sixty and eighty. No very small sporangia as were found in *N. ramosa* were observed in *N. profusa*.

The profusion of extramatrical growth of this species can be traced to the numerous much-branched filaments that arise from five to eighteen main "trunks" originating at a single rhizoidal system. Also, the filament distal to an intercalary zoosporangium is wide and branches one or more times before narrowing to a blunt end or being terminated by another zoosporangium. Thalli bearing predominantly mature sporangia give the impression that

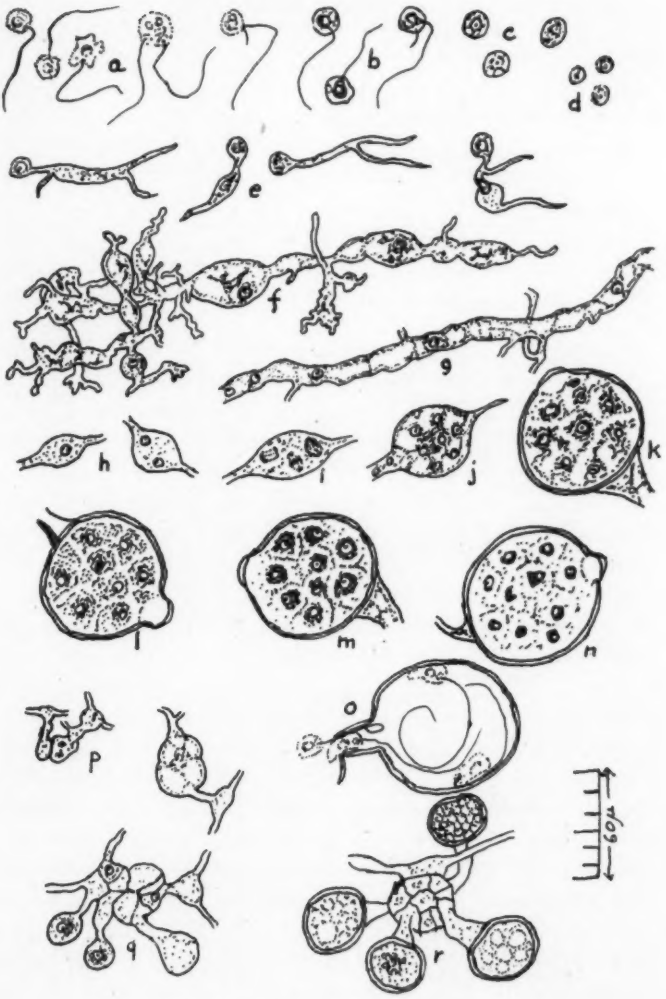


FIG. 2. *Nowakowskiella profusa.*

TABLE OF MEASUREMENTS FOR STRAINS STUDIED
(expressed in microns)

| Structure | *N. ramosa* | *N. profusa* |
|---|---|---|
| *Reproductive Structures* | | |
| Zoospore | | |
| Diameter of spherical spore | 4.85–9.7 | 3.9–4.7 |
| Dimensions of amoeboid spore | 5.82–7.7× 9.7–15.5 | 3.5–7.7× 3.5–4.5 |
| Length of flagellum | 24–35 | 20–30 |
| Diameter of nuclear cap | 2.0–2.3 | 1.5–2.0 |
| Diameter of oil globule | 2.5–3.0 | 1.0–2.0 |
| Zoosporangium | | |
| Diameter of spherical sporangium | 27.16–31.37 | 17.0–40.0 |
| Dimensions of sporangium other than spherical | 35.9–56.3× 37.0–77.6 | 11–20× 40–50 |
| Length of papilla | | |
| Usual form | 2.0–15.0 | 4.0–6.0 |
| Long variety | 50.0–80.0 | up to 30 |
| Diameter of papilla | 4.5–10.0 | 4.0–8.0 |
| Diameter of operculum | 2.91–8.7 | 3.0–4.0 |
| Thickness of operculum | 0.9–1.9 | 0.6–1.0 |
| Diameter of sub-opercular space | 1.8–5.0 | 1.5–6.0 |
| Thickness of wall | 0.6–1.3 | 0.6–1.0 |
| Diameter of nucleus | 1.5–2.0 | 1.5–2.0 |
| Subsporangial Swelling | | |
| Diameter | 3.9–13.6 | 0 or 2.0–6.5 |
| Length | 6.8–15.5 | 7.0–16.0 |
| Thickness of wall | 0.9 | 0.5–0.7 |
| Diameter of subtending filament | 2.0–5.0 | 1.5–4.0 |
| Resting Cell | | |
| Diameter | 7.5–15.0 | — |
| Thickness of wall | 0.5–0.6 | — |
| Diameter of nucleus | 2.0–5.0 | — |
| Flexuous Filaments | | |
| Swelling | | |
| Diameter of cell | | |
| Near vegetative center | 7.0–10.0 | 2.5–5.0 |
| In distal portion | 2.8–7.0 | 2.0–2.5 |
| Length of cell | | |
| Near vegetative center | 7.0–12.0 | 6.0–11.0 |
| In distal portion | 11.0–35.0 | 11.5–30.0 |
| Thickness of wall | 0.2 or less | 0.2 or less |
| Diameter of nucleus | 1.5–2.0 | 1.5–2.0 |
| Isthmus | | |
| Length | | |
| Near vegetative center | 2.0–25.0 | 4.0–30.0 |
| In distal portion | 5.0–60.0 | 4.0–40.0 |
| Diameter | | |
| Near vegetative center | 1.7–4.0 | 1.5–2.5 |
| In distal portion | 1.5–5.0 | 2.0–4.0 |
| Thickness of wall | 0.9 | 0.5–0.7 |
| Diameter of nucleus | 1.5–2.5 | 1.5–2.0 |
| *Vegetative Center* | | |
| Swelling | | |
| Diameter of cell | 4.0–20.0 | 3.0–12.0 |
| Length of cell | 4.0–30.0 | 4.0–24.0 |
| Diameter of globules | 0.2–3.0 | 0.2–2.0 |
| Thickness of wall | 0.2 or less | 0.2 or less |
| Diameter of nucleus | 1.5–2.0 | 2.0 |

TABLE OF MEASUREMENTS FOR STRAINS STUDIED—*Continued*

| Structure | *N. ramosa* | *N. profusa* |
|---|---|---|
| Isthmus | | |
| Length | 0.0–7.0 | 0.0–6.0 |
| Diameter | 0.5–1.5 | 0.5–2.0 |
| Thickness of wall | 0.2 or less | 0.2 or less |
| Rhizoids | | |
| Length | | |
| Branched | 9.0–60.0 | 9.0–48.0 |
| Unbranched | 2.0–30.0 | 2.0–20.0 |
| Diameter | | |
| Branched | 0.5–1.5 | 0.5–2.0 |
| Unbranched | 0.5–0.8 | 0.5–1.0 |
| *Extent of Vegetative Portion* | 35×50–35×200 | 40×40–16×160 |
| *Extent of Reproductive Portion* | up to 2 *cm.* | up to 2.5 *mm.* |

these reproductive bodies are most often intercalary, although some may be formed on the tips of short lateral branches.

The present strain of *N. profusa,* as Karling's (**1941**) original strain, rarely has subsporangial swellings. If they do occur, they are nearly spherical and are constricted at the cross-septum between the swelling and the sporangium. It is not uncommon to find a short tenuous filament below the sporangium leading from an intercalary swelling as in *N. elongata* (Karling, **1944a**). The portions of the filament above and below the cross-septum continue to enlarge, the incipient sporangium the more rapidly, in such a way as to leave the constriction between two unequal-sized spherical swellings.

The behavior of the cytoplasm in the developing zoosporangia and the spherical subsporangial swellings, if present, is very similar to that in the homologous structures in *N. ramosa.* However, the loss of nuclei and the increase in vacuolation in the filaments which support the sporangia is less pronounced in *N. profusa* than in *N. ramosa.* Possibly the presence of more intercalary swellings in the latter is a factor affecting the presence of nuclei in the non-swollen filaments.

True internal proliferation of the zoosporangium has not been observed by the author in either *N. ramosa* or *N. profusa;* but an unusual type of proliferation is common in the latter. As the culture becomes older, the swollen or non-swollen filament above and below the original sporangium may enlarge and become divided into a chain of two to eight secondary zoosporangia. If the swell-

ings form close together their adjacent walls are flattened where they meet in enlarging. Less often there is a short isthmus between two sporangia in a chain. In either case, each individual sporangium lays down a complete thickened wall. Except for the progressive formation and the double adjacent walls, these chains of sporangia resemble the multiseptate zoosporangia of *N. elongata.* After the chains of secondary sporangia form, the filaments appear emptied of nuclei and cytoplasm. These reproductive structures do not always mature and discharge their spores at the same time, even though they may be adjacent on the same filament.

## DISCUSSION

The long extramatrical filaments which arise from the vegetative portion of the thallus, often in great enough numbers to obscure it, are referred to here, collectively, as the "reproductive portion" because each of these filaments may potentially bear intercalary or terminal zoosporangia or, in *N. ramosa,* resting cells. In both the *N. ramosa* and *N. profusa* strains studied the "vegetative" and "reproductive" portions of the thallus are distinct from one another in external and internal structure. The vegetative portion as a separate unit superficially resembles the "rhizomycelium" of the other polycentric chytrids with the nuclei confined to the replicated swellings and the presence of anucleate isthmuses and rhizoids. However, these two species differ from the others in that no reproductive organs are produced from the swellings of the vegetative portion. A possible exception to this is that Karling's (**1941**) original description of *N. profusa* reports the formation of resting bodies formed directly from the intramatrical swellings. The present strain of *N. profusa* is sterile in this respect. Since reproductive bodies may be formed intramatrically on short filaments which do not extend out of the cells of decaying grass leaves, it might be that the resting bodies described by Karling, as well as those by Whiffen (**1943**) for *N. delica,* were not transformed true vegetative swellings.

The sterile swellings confined to the vegetative portion of *N. ramosa* and *N. profusa* appear to be similar to the intramatrical prosporangial (the term is used here in the sense of Whiffen, **1944**)

swellings such as are found in *Nephrochytrium*, especially those forms which are lobed. Karling (**1938**) suggested that this monocentric genus might be a stage in the evolution of the polycentric mode of development. If this conjecture were acceptable the flexuous filaments on which the reproductive organs are borne would be elongated and branched homologues of the isthmuses between the intramatrical prosporangia and the extramatrical zoosporangia. Such a hypothesis is substantiated to some degree by the occasional formation of a single sporangium close to the substratum on a short filament which extends only from the intramatrical swelling in the adjacent cell of the decaying leaf. The swellings in the extramatrical filaments might be the result of progressive sterilization accompanying the process of elongation.

The term "rhizomycelium" does not seem to characterize adequately the reproductive portions of these two strains. The filaments are specialized outgrowths of the vegetative portion and are not intimately associated with rhizoids in structure or function. Unlike the typical rhizoids of the chytrids, they do not become completely devoid of cytoplasm, nuclei, and food materials during the production of a single zoosporangium; but retain enough to produce secondary, tertiary, and chains of zoosporangia in basipetal succession (especially in *N. profusa* which is lacking numerous swellings in these filaments). Unlike the case in the "rhizomycelium" of *Cladochytrium replicatum*, the nuclei are not confined in the swellings of these sporangium-bearing filaments.

The vegetative portion persists throughout the life of the thallus as an absorptive and storage unit, so that a complete change from a trophocentric to a genocentric thallus does not occur. In this manner the thallus of *Nowakowskiella* resembles the true mycelium of the higher fungi, and continues to digest the substratum and produce asexual spores until the available food supply has been diminished or the concentration of metabolic waste in the medium inhibits growth.

Likewise, similar to the higher fungi, when the culture has aged *N. ramosa* produces fewer asexual spores, and initiates resting cell formation. So far as it has been possible to determine, the production of resting cells in this species is not preceded by nuclear fusion. Karling (**1945c**) refers to the fusion of filaments before the forma-

tion of the pseudoparenchyma as "vegetative anastomosing." Shanor (**1942a**) presented some evidence that a fusion nucleus may be present in the resting cell of *N. hemisphaerospora*. The same might be true of *N. ramosa*, for the cytoplasmogamy in *N. hemisphaerospora* bears some resemblance to the formation of the first cell of the pseudoparenchyma in *N. ramosa*. The formation of "parthenogenetic" resting spores has been reported for the genus *Siphonaria*, a genus which supposedly demonstrates sexuality among the chytrids (Karling, **1945c**).

In general, the two species of *Nowakowskiella* studied do not differ greatly in behavior from that described for other chytrids in regard to the nuclei and cytoplasm during the development of the thallus and the formation of reproductive organs. Such differences as do occur may be primarily due to the specialization of the portions of the thallus and the extensive extramatrical development.

The most pronounced characteristic by which *N. ramosa* and *N. profusa* differ from the other polycentric chytrids is the distinct differentiation of the thallus into vegetative and reproductive portions, both of which persist in a functional state.

As to the relationships of *Nowakowskiella*, little can be ventured until other members of the operculate series of the Chytridiales are investigated more fully. In general habit, except for the intramatrical swellings and in the possible fusion of filaments preceding the formation of resting cells, this genus might suggest an incompletely known phylogenetic series between the Chytridiales and the Zygomycetes. Until much more is known about the trophic mycelium as compared with the reproductive mycelium of the latter and until possible transition forms have been investigated, this hypothesis can be considered merely as an interesting speculation.

## SUMMARY

1. The growth of *Nowakowskiella ramosa* and *N. profusa* is abundant on solid substrata composed primarily of cellulose if the surrounding liquid contains organic decomposition products, such as found in river water, pond water, etc.
2. The natural optimum temperature for the development of *N. ramosa* is between 16° and 18° C. and that of *N. profusa*, 24° and 28° C. Both species may be adapted to cultivation at other temperatures.

3. The cell walls of both species show a predominance of chitin which is mixed with cellulose in the same layer. The opercula are pectinaceous.
4. The behavior of the protoplasm during development of the thallus and formation of the reproductive organs does not differ greatly from that in other chytrids.
5. The flagellum appears to be formed within the sporangium and is connected with the nucleus of the zoospore by a rhizoplast near one of the points of a tilted nuclear cap.
6. The center of growth in the developing thallus is early transferred from the spore case to a swelling in the germination tube.
7. The mature thallus is divided into two distinct portions which function concurrently, the vegetative portion and the reproductive portion.
8. The complete thallus is not adequately characterized by the term "rhizomycelium" which has been applied to the thalli of the polycentric chytrids.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Distinguished Professor of Botany Ernest A. Bessey of Michigan State College for his helpful guidance and suggestions in the completion of the investigations and his careful correction of the manuscript. Thanks are also given to Professor Richard de Zeeuw for his advice in methods of staining and mounting specimens.

## EXPLANATION OF FIGURES

FIG. 1. *Nowakowskiella ramosa*. *a*. Living zoospores. *b*. Stained zoospores. *c*. Typical spores after having become quiescent. *d*. Miniature nonflagellate spores. *e*. Germination of spore. *f*. Vegetative portion of thallus. *g*. Flexuous filaments. *h*. Uni- and binucleate zoosporangium initials. *i*. Mitotic division in a trinucleate zoosporangium initial. *j*. Sporangium initial with granular strands and beginning of cross-septum. *k*. Sporangium, with mature wall and operculum, in which vacuolar cleavage has begun. *l*. Sporangium showing complete cleavage. *m*. Sporangium showing aggregation of granules about nuclei. *n*. Mature sporangium showing compact nuclear caps. *o*. Release of zoospores, showing flagella in zoosporangium and one spore held by a looped flagellum. *p*. Early stages in plasmogamy, preceding formation of the pseudoparenchyma. *q*. Pseudoparenchyma with resting cell initials. *r*. Mature resting cells.

FIG. 2. *Nowakowskiella profusa*. *a*. Living zoospores. *b*. Stained zoospores. *c*. Germination of spore. *d*. Vegetative portion of thallus. *e*. Flexuous filament showing pseudosepta at constrictions. *f*. Typical flexuous filament. *g*. Stages in zoosporogenesis drawn from living material. *h*. Stages in zoosporogenesis drawn from stained material. *i*. Reduced sketches of shapes of proliferating and elongate zoosporangia. *j*. Late stage in zoospore escape, showing looped flagella and hyaline pseudopodia.

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# MYRIOGONIUM, A NEW GENUS AMONG SIMPLIFIED ASCOMYCETES [1]

ROY F. CAIN [2]

(WITH 56 FIGURES)

Because of their supposed significance in the evolution of the Ascomycetes the non-ascocarpous species of this group are of special interest. While examining a specimen of *Odontia sudans* (Alb. & Schw., ex Fr.) Bres. collected July 29, 1933 by the late Professor V. Litschauer of Innsbruck, Austria, the writer noticed a peculiar associated Ascomycete. In habit this fungus somewhat resembles *Helicogonium Jacksonii* which was described by W. L. White (**4**) from collections made on *Corticium microsporum* (Karst.) Bourd. & Galz. in Ontario. More detailed study showed that it is quite different and apparently undescribed.

## **Myriogonium** gen. nov.

Ascocarpio nullo; mycelio vegetativo parco, hyphis ramosis, septatis; cellulis terminalibus (gametangiis) binis conjungentibus; cellula ab fusione creta ascos multos in cymbiformi fasciculo gignente; ascis octosporis, elongatis, sessilibus, basi septo cum hamo separatis, apice incrassato, obtuso, in maturitate aperto; ascosporis hyalinis, unicellularibus, elongato-ovatis.

Ascocarp lacking; vegetative mycelium scanty, the hyphae branched, septate, the terminal cells acting as gametangia but not distinctly differentiated; fusion cell forming several asci in a unilaterally cymose cluster; asci eight-spored, elongate, sessile, separated from fusion cell by a septum with hook, the apex thickened, obtuse, liberating spores through an opening at apex; ascospores hyaline, one-celled, elongate-ovate.

[1] Contribution from the Department of Botany, University of Toronto.

This study was carried out with the assistance of a grant in aid of research provided by the University of Toronto.

[2] The author is greatly indebted to Professor H. S. Jackson for suggestions and criticism of the manuscript and to Miss D. F. Vick for copying and inking the drawings.

**Myriogonium Odontiae** sp. nov.

Ascocarpio nullo; mycelio vegetativo parco, plerumque in hymenio hospitis sed in subhymenium extendente; hyphis hyalinis, flexuosis, profunde ramosis, propinque septatis, tenui tunicatis, ad septum constrictis, cellulis 5.0–10 × 1.0–2.5 μ; cellulis terminalibus subincrassatis, binis conjungentibus, ambabus rarenter in duabus ramis ejusdem hyphae cretis sed saepissime in duabus ramis dissimilium hypharum gestis; cellula ab fusione creta sine septo tenente et apice tumorem gignente; asco ex cellula subultima typici hami gerente; cellula ultima cum cellula ad basem conjungente; hac cellula hamos et ascos continenter gerente; ascis initio ovatis, tum elongatis, maturitate late clavatis vel subcylindraceis, sessilibus, octosporis, 18–25 × 5–7 μ, apice obtuso-rotundato, foramine lato ad ascosporarum emissionem apertante, incolorabili iodi ope; sine paraphysibus; ascosporis hyalinis, elongato-obovatis vel prope ellipsoideis, sursum latius rotundatis, 4.5–6.0 × 1.2–1.7 μ, unicellularibus, oblique distichis.

Ascocarp lacking; vegetative mycelium scanty, mostly in hymenium of its host but extending into subhymenium; hyphae hyaline, flexuous, profusely branched, closely septate, thin-walled, constricted at the septa, with cells measuring 5.0–10 × 1.0–2.5 μ, terminal cells becoming somewhat swollen and fusing in pairs, usually from separate but occasionally from the same branching system; two fusing cells remain continuous and grow out by means of a swelling at apex of one of fusing cells; an ascus is formed from penultimate cell of a typical crozier; the ultimate cell fuses with basal cell which remains continuous with fusion cell; this continuing to produce croziers by proliferation in the region of the ultimate cell so that a small group of asci are formed each from a penultimate cell in a unilaterally cymose cluster; asci at first ovate, elongating, at maturity broadly clavate to subcylindric, sessile, eight-spored, 18–25 × 5–7 μ, the apex obtuse, rounded, opening to discharge ascospores through a wide pore at apex, not colored by iodine, paraphyses lacking; ascospores hyaline, elongate-obovate to nearly ellipsoid, more broadly rounded at upper end, 4.5–6.0 × 1.2–1.7 μ, one-celled, obliquely biseriate or somewhat irregularly arranged.

On *Odontia sudans* (Alb. & Schw., ex Fr.) Bres. on decaying wood of coniferous tree. Hochmoos bei Platzl in der Leutasch, Tirol. July 29, 1933. *V. Litschauer* (77). **Type** (in University of Toronto Cryptogamic Herbarium).

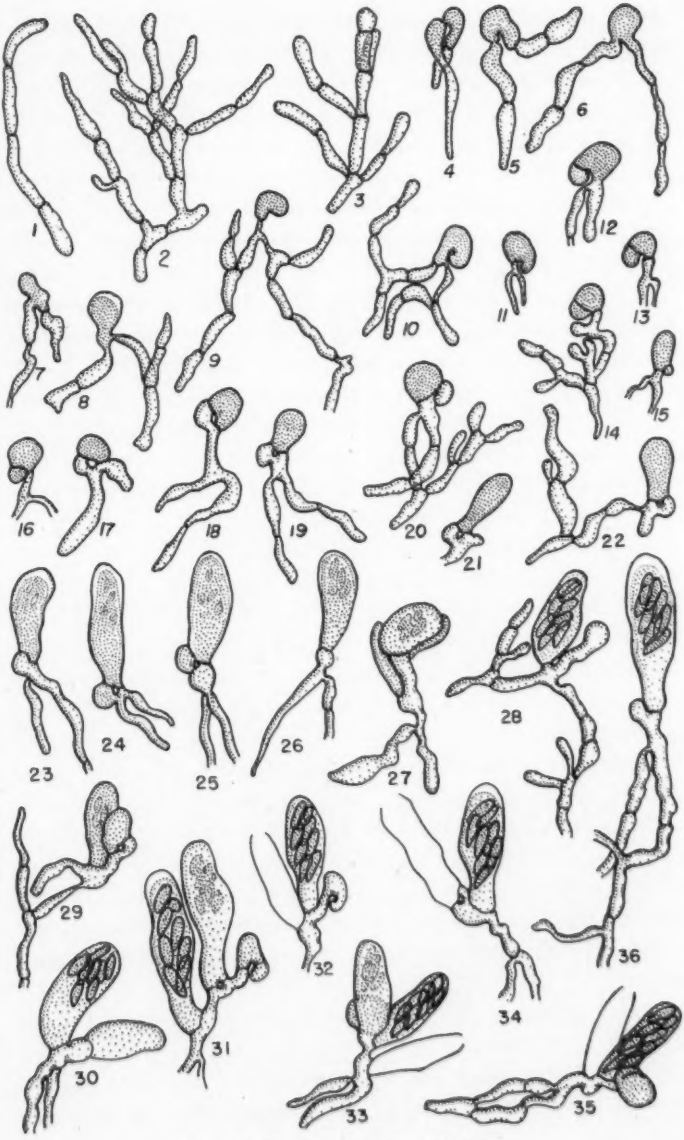
From the location of the asci the fungus appears to be parasitic on the *Odontia,* and covers a little more than half of the area included in the collection studied. There is no change in the appearance of the *Odontia* due to the presence of the Ascomycete

that is visible to the naked eye or evident under a high power wide field binocular microscope. Asci have not been found in the extreme marginal area but in some places occur a few millimeters away. They become more abundant in the older areas and in some places the hymenium of the Basidiomycete is entirely replaced by the parasite. In the newly infected areas asci are found scattered in the same field as basidia bearing normal basidiospores. Some areas of mature hymenium several centimeters in diameter are entirely free from asci.

There is nothing in the appearance of the specimen to suggest that the Ascomycete might have developed during or subsequently to drying of the fresh material. Only the one packet of the collection has been available for examination. It seems probable that the portion of the same collection in Litschauer's herbarium would also bear the Ascomycete.

The ascomycetous hyphae (FIGS. 1–3, 54, 56) are entirely different from the clamped hyphae of the host (FIG. 56) in being narrower with short cells constricted at the septa. These could not be traced beyond the subhymenium of the host and are found most abundantly in the hymenium (FIG. 56). No connection between the two sets of hyphae could be found. The young branching hyphae are most abundant in the newly infected areas. In the older portions these are largely replaced by the clusters of asci with the fusion cell and two chains consisting of three or four vegetative cells each. The hyphae in the hymenium appear to arise at right angles from more elongate hyphae with less frequent branching located in the subhymenium (FIG. 54).

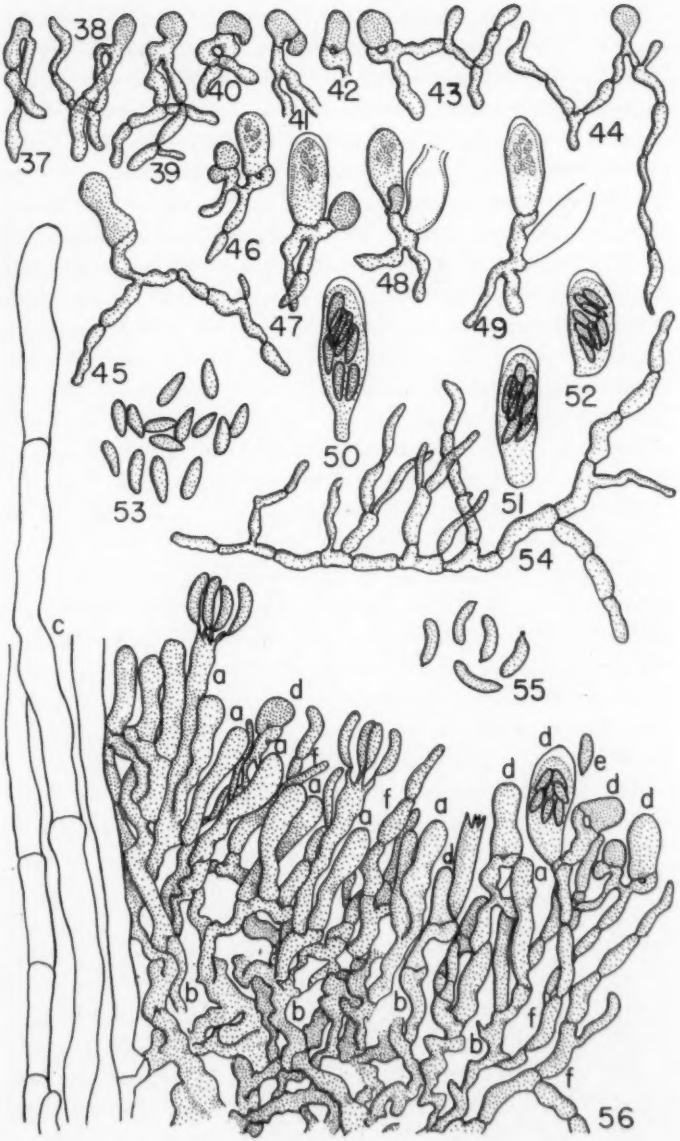
The fungus appears to be distinctly different from and perhaps not immediately related to *Helicogonium Jacksonii* in lacking anything that can be interpreted as ascogonia and antheridia. Fusion cells are produced by the growing together of two terminal cells of the branches (FIGS. 4–7, 37–39). These are not separated from the two fusing gametangia by septa so that they appear with a two-pronged base (FIGS. 5–6) and are produced in great abundance. Apparently any of the terminal cells of the branched hyphae may function as gametangia. Fusion usually takes place between cells of different branching systems (FIGS. 6, 9, 10, 28, 39, 44) but a few have been found in which the two gametangia could be traced




FIGS. 1–36. *Myriogonium Odontiae.*

back to the same branch one or two cells below (FIG. 35). Gametangia about to fuse (FIG. 4) or which have just united (FIGS. 5, 37) are difficult to find. In the few cases that were seen they were distinctly swollen, the one sometimes curved to meet the other. All stages of development subsequent to fusion could be found in abundance. These show no evidence of any swelling or of any appreciable differentiation between the two fusing cells (FIG. 5). A distinct constriction is usually seen where the one has fused with the other. The two fusing cells remain with a continuous cavity. Near the point of fusion a swelling takes place in the wall of one of the original gametangia which is typically separated from the other by a narrow constriction, but no septum is developed (FIGS. 5, 37). This swelling becomes ovate or subglobose (FIGS. 6–8, 38–39) and then elongates and curves into a somewhat reniform structure (FIGS. 10–11, 40), and develops into a typical crozier. A septum cuts off a basal cell which remains continuous with the forked fusion cell (FIGS. 11, 40). A second septum cuts off an ultimate cell (FIGS. 12–16, 41) which fuses with the basal cell (FIGS. 17–18, 42). The penultimate cell enlarges on the distal side (FIGS. 19–22, 42–45) and always develops into an ascus (FIGS. 23–26). This is followed by an outgrowth in the region of the ultimate cell (FIG. 28) which develops into a crozier (FIG. 29) which produces a second ascus (FIG. 34) in a manner similar to the first. This process of crozier formation is continued so that there is produced a unilaterally cymose cluster of asci which mature in succession (FIGS. 31–32). Each cluster usually has a crozier, a partially developed ascus, a mature ascus and one or two empty ones. Swellings in the basal cell below often show that several other asci have matured and disintegrated (FIG. 34). There are no paraphyses and apparently all of the terminal cells of the vertical branches are used in the production of gametangia as few or none are left in the older portions.

Such a system of ascus formation is adapted to the gradual and continuous development of asci and in this case probably only limited by the exhaustion of the food supply of the host. By this method the penultimate cell of each crozier always develops into an ascus.




FIGS. 37–56. *Myriogonium Odontiae.*

This method of development is similar to that described by Rogers (3) for basidial proliferation in *Sebacina prolifera* Rogers. Here the cell which corresponds to the penultimate cell of a crozier always develops into a basidium while renewed growth takes place from the stalk in the region of the beak. In this manner an indefinite series of basidia maturing in succession is formed and probably limited only by failure of food supply or unfavorable weather conditions. This is in marked contrast to the method of ascus formation by croziers in a species such as *Aspergillus Fischeri* Wehmer. In this species according to Olive (2) there is a proliferation of ascogenous cells by means of croziers which develop from both the penultimate cells and the crozier tip. The distinction here is that the penultimate cells of the croziers develop into new croziers instead of asci. Not until the ultimate growth of the mass of ascogenous hyphae has been reached do the penultimate cells cease forming croziers and develop into asci. Such a system results in the more or less simultaneous development of all of the asci within the ascocarp.

According to Rogers (3) in *Pyronema confluens* (Pers.) Tul. the ascogenous hyphae in the early stages of their development proliferate by the formation of a new crozier from the penultimate cell of the previous crozier. Only in later development is this process terminated by the formation of an ascus instead of a crozier from the penultimate cell. At this stage new croziers are produced from the region of the basal or ultimate cells.

Many who adhere to the doctrine of the evolution of the Ascomycetes from some simple representative such as *Dipodascus* to the more complex types with elaborate ascocarps, may consider *Myriogonium Odontiae* as a representative in the early series of such an evolution. No such significance is attributed by the author to this fungus. On the contrary, it is considered as a highly specialized member in a terminal series of morphologically reduced forms, the reduction in morphology being associated with its highly specialized habitat. While its actual relationships are uncertain its ancestral forms are to be sought among the more elaborately organized fungi, possibly among the inoperculate Discomycetes. It seems quite possible that *Helicogonium* may also represent a

simplified form derived from some higher Ascomycete line rather than belonging among the Hemiascomycetes.

*Myriogonium* differs from *Helicogonium* in several important respects, especially in having one-celled ascospores which do not bud in the ascus, in lacking ascogonia and antheridia and in possessing croziers. In *Myriogonium* fusion takes place between hyphal tips, or only very slightly specialized branches of the hyphae. This may represent the result of a reduction from some more elaborate type of gametangia or might be interpreted as hyphal anastomoses where fructification results at each point of fusion. The fact that *Helicogonium* lacks croziers cannot be taken to indicate a complete lack of relationship. Species in the same genus may or may not have croziers. Emmons (**1**) has shown that in *Thielavia terricola* (Gilman and Abbott) Emmons the asci arise from the penultimate cell of a crozier while in *Thielavia Sepedonium* Emmons they arise as simple side branches from the ascogenous hyphae without crozier formation. A similar situation is reported by White (**5**) for *Helotium*, a genus of inoperculate Discomycetes. In *Helotium albidum* (Rob., ex Desm.) Pat. and *H. Dearnessii* (Ell. & Ev.) White the asci do not originate from croziers. In such species as *H. citrinulum* Karsten, *H. midlandensis* White, *H. erraticum* White, *H. scutula* (Pers., ex Fr.) Karst. var. *fucatum* (Phill.) Rehm and *H. gemmarum* Boud. the asci develop from croziers.

Within the Basidiomycetes there are many related groups of species some of which have clamped hyphae and some lack clamps. It is generally considered, by students of this group, that the species lacking clamps have been evolved from the clamp-bearing species. In a similar manner the ascomycetous species lacking croziers have probably developed from forms in which they are present.

It is perhaps worth while to point out that the "fungus" here described is not a single individual as it is found on its host nor a single generation. The vegetative mycelium is the haploid generation and consists of one individual plant (if from a single spore) or several individual plants growing together (if from two or more spores). Each cluster of asci together with the fusion cell from which it arises represents an individual plant of the dikaryotic

diploid generation. Each ascospore represents the beginning of a new haploid individual.

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## EXPLANATION OF FIGURES

All figures were drawn with the aid of a camera lucida from preparations of crushed material mounted in potassium hydroxide-phloxine. *Figs. 1–36* drawn at a magnification of 1600 and reduced in reproduction to 1070. *Figs. 37–56* drawn at a magnification of 2100 and reduced to 1120.

*Figs. 1–3.* Branched hyphae. *Fig. 4.* Two separate branches just before fusion of the terminal cells or gametangia. *Fig. 5.* Fusion cell just after fertilization. The upper part of the fusion cell has produced a small swelling, the early stage in the formation of the ascus hook. *Figs. 6–9.* Early stages in the formation of the outgrowth from the fusion cell. *Figs. 10–11.* The outgrowth has elongated and curved into a kidney-shaped structure, the ascus hook. *Fig. 12.* The hook has cut off a terminal cell. *Figs. 13–16.* Two septa have formed to divide the terminal or ultimate cell and the penultimate cell from the fusion cell. *Figs. 17–18.* The terminal cell has fused with the basal or fusion cell. *Figs. 19–26.* Stages in the development of the first ascus from the penultimate cell. *Fig. 27.* Third ascus has begun to form. *Fig. 28.* The ultimate cell of the first hook, after uniting with fusion cell, has grown out prior to the formation of the second hook. *Fig. 29.* The second ascus has begun to enlarge from the penultimate cell of the second ascus. *Fig. 30.* The first ascus has mature spores and the second one partly developed. *Fig. 31.* The ultimate cell of the third hook is about to fuse with the basal cell which remains continuous with the basal cells of the second and first hooks as well as the fusion cell. *Figs. 32–35.* The first ascus has discharged its ascospores through a pore in the apex. *Fig. 36.* A mature ascus with the fusion cell formed from two gametangia, the terminal cells of two branches borne on separate hyphal systems. In *Fig. 35* the gametangia were the terminal cells of two-celled branches originating on the same hyphal cell.

*Fig. 37.* Two separate branches with two cells each; the terminal ones (gametangia) have fused. *Figs. 38–39, 44.* Development of the swelling from the fusion cell. *Fig. 40.* The basal cell (which is continuous with the fusion cell) is cut off by a septum. *Figs. 41–43.* The penultimate cell is separated by septa from the basal and terminal cells. *Fig. 45.* Enlargement of the penultimate cell of the first hook to form the first ascus. *Figs. 46–47.* The second ascus beginning to develop and spores being delimited in the first. *Figs. 48, 49.* Empty ascus with others in various stages of development. *Figs. 50–52.* Asci with mature spores and thick-walled apex. *Fig. 53.* Ascospores after discharge from asci. *Fig. 54.* Vertical branching hyphae with terminal gametangia growing from a vertical vegetative hypha. *Fig. 55.* Basidiospores of *Odontia sudans*. *Fig. 56.* Section of *Odontia sudans* taken near the apex of a tooth of fruiting body. *a.* young basidia; *b.* hyphae bearing basidia; *c.* cystidia of host forming axis of tooth and projecting at apex; *d.* asci in various stages of development; *e.* ascospores; *f.* branched hyphae of *Myriogonium*.

# MARTENSELLA CORTICII THAXTER AND ITS DISTRIBUTION [1]

H. S. Jackson and E. R. Dearden

(with 11 figures)

Incidental to the study and identification of several thousand Canadian collections of corticioid Basidiomycetes during the past fifteen years in the mycological laboratory of the University of Toronto, a number of interesting associated fungi have been encountered, some or all of them parasitic on the Basidiomycetes.

One of the first of these to attract attention was found growing on the surface of a fructification of *Corticium bombycinum* (Somm.) Karst. and was described and illustrated by the late Dr. David H. Linder (**4**) as *Spondylocladiella botrytioides,* a new genus and species of dematiaceous Hyphomycete. A second form, found several times growing on *Corticium microsporum* (Karst.) Bourd. & Galz., proved to be a simple Ascomycete producing individual clavate asci scattered among the basidia in the hymenium of the Corticium in such a manner that, when first observed in an immature condition, they were mistaken for the cystidia of a *Peniophora.* The collections of this Ascomycete were finally sent to Dr. W. L. White (**8**) who described the parasite as *Helicogonium Jacksonii,* a new genus and species which he assigned, perhaps incorrectly, to the Hemiascomycetes. *Platygloea Peniophorae* Bourd. & Galz., found associated with fructifications of *Peniophora,* was first reported from North America by Dr. G. W. Martin (**6**), based on Canadian collections sent him from this laboratory. In the same paper Martin also described as new species *Tremella mycophaga* on *Aleurodiscus amorphus* (Pers.) Rabenh. ex Cooke, and *Tremella simplex* Jackson & Martin on an as yet undescribed *Aleurodiscus* on *Thuja occidentalis,* both from Ontario. Recently the

[1] Contribution from the Department of Botany, University of Toronto.

This study was carried out with the assistance of a grant in aid of research provided by the University of Toronto.

senior author (**3**) has described, as a new genus and species, *Trichomonascus mycophagus,* a remarkable Ascomycete of uncertain relationship found growing from the hymenium of *Corticium confluens* Fr. Still more recently Dr. R. F. Cain (**2**) has published, also as a new genus and species, *Myriogonium Odontiae,* another unusual Ascomycete found associated with the hymenium of *Odontia sudans* (Alb. & Schw., ex Fr.) Bres. This was discovered on a collection of the host in the herbarium of the University of Toronto, made in Austria by the late Professor V. Litschauer. The senior author has in manuscript an undescribed species of *Helicobasidium* found growing in relation to a species of *Peniophora.* Several other collections of auriculariaceous fungi growing in association with resupinate Thelephoraceae await further study.

## MARTENSELLA CORTICII Thaxter

In addition to this rather remarkable series of associated fungi, another form was observed first in the fall of 1945 by the junior author, growing on the hymenium of *Corticium radiosum* Fr. This proved to be a Phycomycete, *Martensella Corticii* Thaxter, described as follows in a recent paper by Linder (**5**, p. 59) and assigned to a new family, the Kickxellaceae, of the Mucorales.

"Colonies effuse, forming a minutely and sparsely hirsute layer over the substratum, pale yellowish or 'Cream Color.' Conidiophores simple, hyaline, 1–3-septate, up to 200 $\mu$ long, 5.5–6.5 $\mu$ in diameter and bearing one or two, rarely three, sporocladia. Sporocladia stipitate, the stalk cell 9–12.5 × 4.5 $\mu$, the sporocladia (2)–3–4–(5)-septate, 25–36 $\mu$ long, inflated in the fertile portion where they are 7–10 $\mu$ in diameter, somewhat tapering to the bluntly rounded base and the sharply upturned sterile apex. Phialides 6–8 on the upper surface of the sporocladium, 5–6 × 3.5 $\mu$. Conidia elongate-ellipsoid to subcylindrical, slightly tapering towards the base, rounded towards the apex, 10.5–13 × 3.5–4.5 $\mu$.

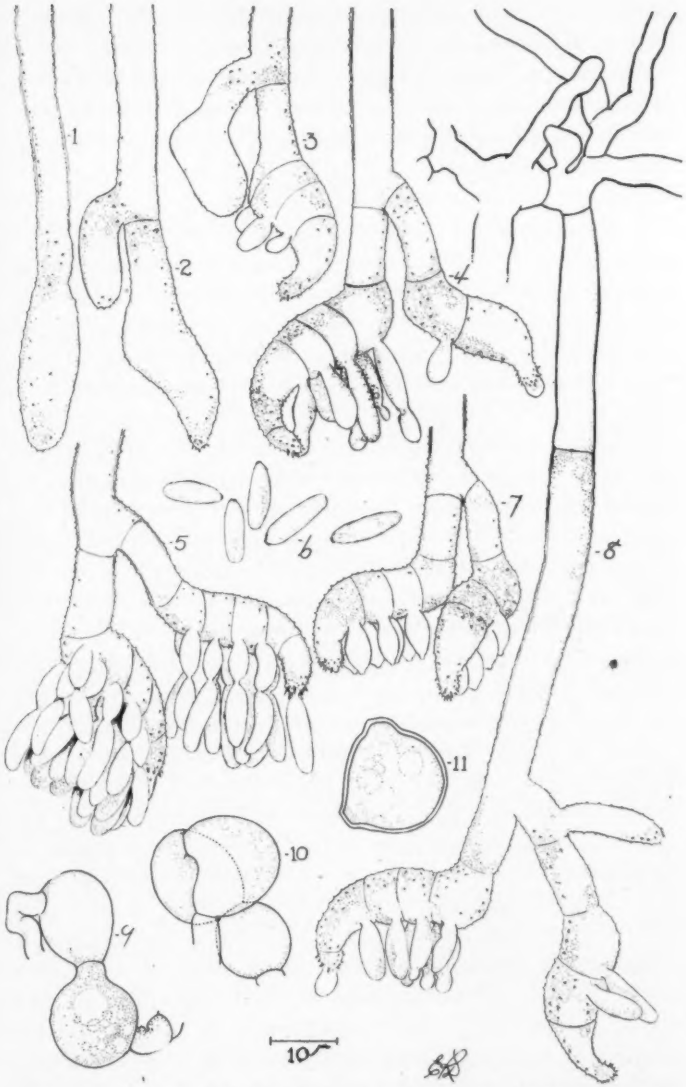
Specimens examined: **New Brunswick,** Campobello, on *Corticium* sp., W. G. Farlow, **type;** New York, Enfield Gorge near Ithaca, on *Corticium alutaceum,* Oct. 18, 1902, G. F. Atkinson."

The above description was checked with the type and other collections. It is not entirely satisfactory, and requires several amendments. Contrary to the description, the apex of the sporo-

cladium is not always sterile but may function as a phialide bearing a single conidium (FIG. 5). As a minor omission, it should be noted that the walls of the aerial portion of this fungus are delicately asperulate, and the apices of the sporocladia echinulate. The conidia and vegetative portions of the fungus are smooth walled.

As a major omission attention should be drawn to the presence of structures, foreign to the host, consistently associated with the conidial stage of the Phycomycete. These are found imbedded within the Basidiomycete and at maturity are subglobose, 10–20 $\mu$ in diam., smooth and hyaline. The majority of these in the type collection have thin walls, but many in other collections have the walls thickened 1.5–2 $\mu$. Produced singly, or more commonly in chains of 2–5 as intercalary swellings on the vegetative hyphae, they are best interpreted as chlamydospores (FIGS. 9–11). While the organic connection between the aerial conidial phase and the chlamydosporic phase of the mycelium has not been traced, the consistent association in over thirty collections of *Martensella Corticii* from widely distributed localities is submitted as convincing evidence of this connection. It should be emphasized that these structures are of an asexual nature. There is no evidence of a union with any other hyphal branch. That they are borne in chains is evidence of their vegetative origin.

In this connection, however, attention should be drawn to the zygospores described and illustrated by Linder for a related species, *Coemansia aciculifera*. This species had been obtained by Thaxter, according to the evidence available, from germinating zygospores found in Sphagnum. The structures which are referred to above as chlamydospores in *M. Corticii* are certainly not to be interpreted as zygospores or as azygospores. Possibly these correspond to what Linder, in his discussion of *C. aciculifera,* refers to as gangliform swellings on the mycelium. Unfortunately he furnished no figure of these structures and does not describe them in any detail but compares them with structures figured by Van Tieghem for *Mortierella tuberosa* V. Tieg. Linder recognizes clearly the uncertainty of the relationship of the fungi of this group to the Zygomycetes when based on the somewhat uncertain evi-




FIGS. 1–11. *Martensella Corticii;* 1–5, stages in the development of conidiophores with primary and secondary sporocladia; 6, conidia; 7, discharged conidiophore; 8, conidiophore with a tertiary sporocladium developing; 9–10, young chlamydospores in chains; 11, a mature chlamydospore.

dence of zygospores and more reasonably places the Kickxellaceae there on comparative morphological grounds.

All attempts to obtain cultures from the conidia have so far resulted in failure. Special media have not yet been used and no attempt to grow the fungus from chlamydospores has been made.

## DISTRIBUTION

It appears from the evidence available that *Martensella Corticii* occurs only on *Corticium radiosum* Fr. and is probably parasitic, perhaps obligately so on that host. The previously unidentified host of the type proves to be that species. *Corticium alutaceum* (Schrad.) Bres., cited by Linder as the host for the single paratype, is synonymous with *C. radiosum* according to Burt (**1**, p. 263).

Following the first recognition of the parasite in Ontario a number of other field collections have been made but most of the records from that province and elsewhere have been obtained by a careful examination of the considerable number of specimens of *C. radiosum* which were already filed in the University of Toronto herbarium. To determine the distribution of *M. Corticii* more accurately all the specimens of *C. radiosum* and of *C. lacteum* Fr. in the Burt and general herbaria at the Farlow Herbarium were examined through the courtesy of Dr. Rolf Singer. *C. lacteum* was included for examination because Burt had confused it with *C. radiosum* (**7**, p. 294). Similarly the collections in the Lloyd Herbarium and in the Herbarium of the Bureau of Plant Industry were loaned by Dr. J. A. Stevenson and the specimens in the Overholts Herbarium and the portion of the Burt collection at the Missouri Botanical Garden were loaned by Drs. F. D. Kern and C. W. Dodge respectively. Five additional records were obtained from these herbaria.

During this survey all collections were examined carefully with a wide field binocular microscope for the hirsute, orbicular, frequently coalescing colonies of the parasite. The characteristic appearance of these colonies overgrowing the perfectly smooth hymenium of the Basidiomycete makes such an examination relatively simple. Subsequently determinations of *M. Corticii* were confirmed by examination with a compound microscope.

The distribution of *M. Corticii* in North America as revealed by these studies is widespread. Some thirty-one collections are deposited in the University of Toronto Herbarium. These records are from four Canadian provinces and seven American states. None of the limited number of European collections of *C. radiosum* available was parasitized. Although at present known only from North America, *M. Corticii* may perhaps be expected wherever its host, a cosmopolitan species, is found.

## COLLECTIONS EXAMINED [2]

**Ontario:** S. of Aurora, Oct. 5, 1934. R. F. Cain, *21570* from *5433;* Sept. 29, 1946. H.S.J., *21565* from *21293;* Benwell swamp, Gobles, Oxford Co., Sept. 24, 1939. R. F. Cain, *21572* from *15074;* S. of Hatchley, Brant Co., Oct. 27, 1934. R. F. Cain, *21573* from *5439;* Sept. 25, 1937. R. F. Cain, *20124* from *12571;* W. of Maple, Oct. 1946. H.S.J., *21566;* Nashville, York Co., Oct. 13, 1945. R. F. Cain, *20121* from *20204;* Ottawa, 1891. J. Macoun, from MoBGH *44640;* Oxtongue Lake, 5 mi. W. of Algonquin Park, Oct. 14, 1940. H.S.J., *21862* from *16423;* N. of Richmond Hill, Sept. 28, 1934. H.S.J., *21861* from *5497;* Oct. 3, 1935. R. F. Cain, *20123* from *8228;* Sept. 29, 1937. H.S.J., *20125* from *12108;* Oct. 14, 1937, H.S.J., *21571* from *12565;* Silver Lake, Frontenac Co., Sept. 1, 1941. R. F. Cain, *20126* from *17805;* Timagami, Aug. 1907. C. G. Lloyd, *20127* from LH *16005;* Paradis' Bay, L. Timagami, Sept. 3, 1936. R. Biggs, *20122* from *10116.*

**Quebec:** Lower St. Lawrence Valley, Oct. 13, 1905. J. Macoun, from MH *87,* in Herb. Burt FH; Indian Lake, near Wilson's Corners, Oct. 12, 1935. I. Mounce from F *6713,* ex Myc. Herb., Ottawa.

**New Brunswick:** Campobello, W. G. Farlow, **Type,** FH.

**British Columbia:** Clearwater, Oct. 4, 1944. D. C. Buckland, from Myc. Herb. Ottawa *16182,* ex V-934 Dom. Lab. Forest Path. Victoria, B. C.

**Connecticut:** Woodbridge, Oct. 2, 1933. J. R. Hansbrough, from USDA, FP *81254;* Branford, Nov. 10, 1933. J. R. Hansbrough, from USDA, FP *84036;* Cockaponset State Forest, Chester, Nov. 21, 1937. H. G. Eno, from USDA, FP *82476;* Nov. 2, 1937. H. G. Eno, from USDA, FP *82479.*

**New York:** Enfield Gorge, near Ithaca, Oct. 18, 1902. G. F. Atkinson, from CU *14101,* **Paratype,** FH; Warrensburg, Oct. 4, 1931. H. D. House, TRT.

[2] Unless otherwise stated, the collection numbers given below are those of the Cryptogamic Herbarium of the University of Toronto (TRT). Where two numbers are given, separated by the word "from," the first is the segregated specimen of *Martensella* and the second that of the host collection.

**Pennsylvania:** State College, Mar. 11, 1918. L. O. Overholts, from OH *4691*, Penn. State Coll. Herb.; Emlenton, Butler Co., Oct. 6, 1934. L. O. Overholts and W. A. Campbell, from OH *19291*, Penn. State Coll. Herb.

**Michigan:** New Richmond, Nov. 22, 1913. C. H. Kauffman, from MoBGH *22870*.

**Arizona:** Santa Catalina, Coronado Nat. Forest, Oct. 15, 1911. G. G. Hedgcock and W. H. Long, from USDA, FP *9787*.

**Utah:** S.E. of Ephraim, Sept. 19, 1902. G. G. Hedgcock, from USDA, FP *3968*.

**Wyoming:** So. Brush Creek Camp Ground, Medicine Bow Nat. Forest, July 25, 1942. S. M. Pady, TRT.

## DISCUSSION

The three genera of the Kickxellaceae fall naturally into two groups; the genus *Kickxella* is characterized by a whorled arrangement of the sporocladia about the apex of the conidiophore whereas the genera *Coemansia* and *Martensella* are characterized by a diffuse arrangement of the sporocladia along the simple or branched conidiophores. Of the two latter genera, according to Linder, the generic separation is based on the orientation of the phialides; along the upper surface of the sporocladia in *Martensella* and on the lower surface in *Coemansia*. In placing *M. Corticii* in *Martensella* rather than *Coemansia* the orientation of the host to its substrate was apparently not considered. Fructifications of resupinate Basidiomycetes are commonly found in nature on the lower surface of their substrata. If we are to evaluate this factor, it is evident that the aerial portions of a parasite colonizing a Corticium must project downwards. It follows then that the conidia and phialides of *M. Corticii* are pendant from the downward projecting conidiophores and are actually arranged along the lower surface of the sporocladia. It may be argued that this arrangement is simply an adaptation for purposes of dissemination of the conidia, and may be interpreted as of no more significance than a geotropic response.

The method of proliferation of sporocladia in *M. Corticii* (FIGS. 1–5, 8) offers further evidence of the close relationship to species placed in *Coemansia*. Conidiophores of *M. Corticii* bear normally two or rarely three sporocladia. The primary sporocladium develops from a terminal enlargement of the conidiophore (FIG. 1).

This develops asymmetrically and becomes separated by a septum from the supporting conidiophore which then proliferates laterally immediately behind the septum (FIG. 2). A secondary sporocladium develops at the apex of the lateral outgrowth and is also separated by a septum from the supporting conidiophore (FIGS. 3–4). A tertiary sporocladium is occasionally proliferated from immediately behind the secondary one. Further development of the sporocladia follows a common pattern. Each becomes 2–4-septate and produces 2–4 finger-like phialides from all but the first and last cells. These projecting phialides in turn produced conidia as apical swellings. The first cell of each sporocladium is always sterile, the last one produces a single conidium directly from the sharply curved apex or may be sterile. Should the conidiophores continue proliferation, more complicated forms would be evolved with typically more than two or three sporocladia arranged diffusely along the axis of the conidiophore. The majority if not all species of *Coemansia* develop in such a pattern.

Two problems are presented after consideration of *M. Corticii* and forms closely related to it—the first a question involving the generic position of this fungus—the second a question concerning the advisability of separating *Coemansia* from *Martensella*. Following Linder's interpretation of the two genera *Martensella* and *Coemansia sensu stricto, M. Corticii* is properly a *Coemansia*. This decision involved a simple consideration of the effect produced by the orientation of the host upon the fungus. In the final analysis it was seen that the phialides in nature actually are produced from the lower surface of the sporocladia.

The second problem is not dismissed so readily. *Martensella*, erected in 1863 by Coemans for a single species *M. pectinata*, now known only from the author's description, antedates *Coemansia* Van Tieghem & LeMonnier (1873) by ten years. Until the recent description of *M. Corticii*, it remained monotypic. The separation of the two genera as based on the orientation of the phialides may be artificial and may be conditioned by the position of the fungus on its substrate. In any species with a diffuse arrangement of the sporocladia the phialides, depending upon the angle of branching between the sporocladium and conidiophore axis, may be pointed in any direction. Such a nomenclatorial

problem is beyond the scope of this paper and for our present purpose it seems best to follow Linder's treatment. Future students of the group, however, may find it desirable to treat *Coemansia* as a synonym of *Martensella*, or perhaps to provide for the conservation of *Coemansia*.

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CANADA

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# SOME LEAF SPOT FUNGI ON WESTERN GRAMINEAE—II [1]

RODERICK SPRAGUE [2]

(WITH 2 FIGURES)

In addition to previous reports (**6–12**), more fungi that cause leaf spots, or are associated with diseased leaves, of grasses growing in the western United States are discussed or described in this paper. Type material and representative collections are filed in the Mycological Collections, U.S.D.A., Beltsville, Md. Some of the specimens are duplicated in the herbarium of the Dept. of Plant Pathology, Washington State College, Pullman, Wash., and in the Mycological Herbarium, Dept of Botany, Oregon State College, Corvallis, Ore. These studies have been made at the Northern Great Plains Field Station, Mandan, N. Dak.

## **Spermospora** gen. nov.

Conidiis subulatis v. subulati-filiformibus, apicibus elongato-filiformibus, hyalinis, septatis, maculicolis.

Typus: *Cercosporella subulata* Sprague.

Conidia subulate to subulate-filiform, apical cell appearing appendage-like; hyaline, septate, borne superficially on evident conidiophores in spots. A member of the Moniliales and the Moniliaceae.

[1] Cooperative investigations between the Divisions of Cereal Crops and Diseases, Forage Crops and Diseases, and Soils, Fertilizers and Irrigation Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration; and the Nursery Division, Soil Conservation Service, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station.

[2] Formerly pathologist, Division of Cereal Crops and Diseases, and collaborator, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering.

On the basis of continued study of a number of collections, the fungus *Cercosporella subulata* (**6**) is made the type of the new genus. Accordingly, the name of the fungus becomes **Spermospora subulata** (Sprague) comb. nov. The diagnostic characters of *Spermospora* and *Cercosporella* are as follows:

1. Conidia more or less broadly filiform, hyaline, septate—*Cercosporella.*
2. Conidia subulate or narrow-subulate with distal cell elongated into a narrow whip-like tip, hyaline, septate—*Spermospora.*

*Spermospora* belongs, with *Cercosporella,* in section Scolecosporae of the Moniliaceae (Moniliales). The sperm-like shape of the spores of *S. subulata* is so distinct that it seems desirable to separate this species from *Cercosporella.*

*Spermospora subulata* was originally found on an herbarium specimen of *Melica subulata* (Griseb.) Scribn. from eastern Oregon (**6**). Although this fungus is definitely parasitic, causing severe leaf scald, it remains somewhat of a mystery why it is never abundant. It has been collected as far east as the Big Horn Mts., Wyo., by George W. Fischer, on *Melica bulbosa* Geyer (B.P.I. *80, 114*). The writer found the same parasite on an alpine form of *Deschampsia caespitosa* (L.) Beauv. in Quad Creek meadow at Beartooth Pass, Mont. (B.P.I. *80,304*), at an elevation of about 10,000 feet. In 1937 he also collected a small quantity of it on *Festuca rubra* L. west of Halsey, Ore., and near King's Valley east of Wren, Ore. John Hardison sent additional material from Granger, Ore., in 1945. This latter material, also on *F. rubra,* differs in some ways from the type. The spores (FIG. 1, *A*) are 3-septate instead of being typically 2-septate. The extra septum is usually formed at the base of the particularly appendage-like distal cell. This cell has the appearance of being partly germinated. The overall size of the spores is 33–55 × 3.3–4.3 $\mu$ whereas the type on *Melica* has spores 20–35 × 2.5–4.3 $\mu$ (**6**, FIG. 2, *A*). The collection on *Deschampsia* contains spores similar in size. They are faintly yellowish, 2–3-septate and 33–47 × 3–4.5 $\mu$ (FIG. 1, *B*). They are borne in scald-like obscure lesions, typical of the species.

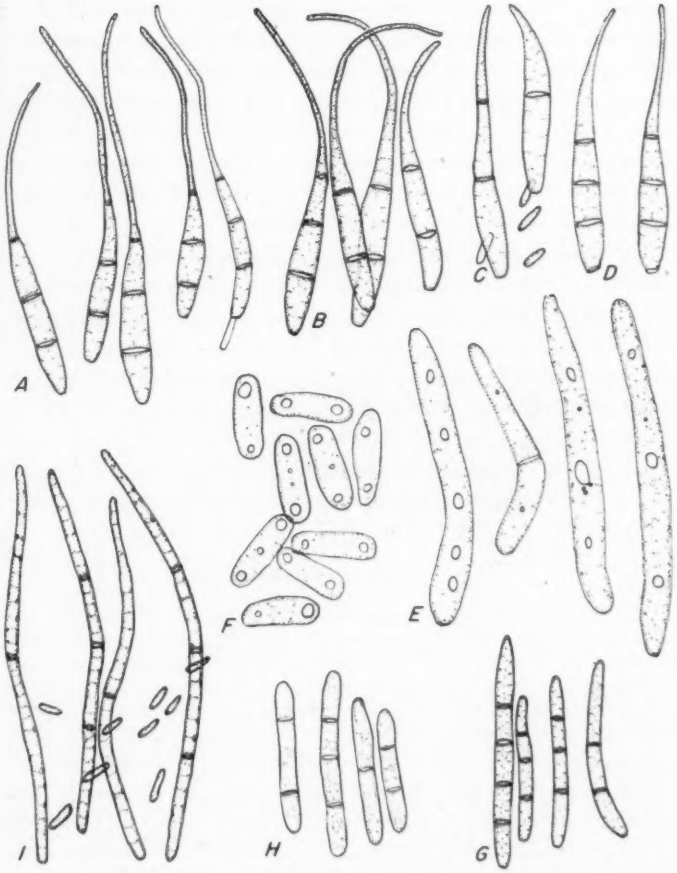


FIG. 1. Spores of leaf spot fungi on western grasses.

A pure culture was obtained from spores on *Festuca rubra* collected near Halsey, Ore., in 1937. The fungus grew slowly on potato-dextrose agar. The colony was felty, depressed, fawn colored and at first it was sterile. Later a few spores, macrospores and microspores, were produced in the refrigerator at 40° F., the macrospores (FIG. 1, *C*) being similar to those formed on the host (FIG. 1, *D*). The macrospores in pure culture measured 35–50 × 3.4–4.6 μ and the microspores, 4–6 × 1.5–2.0 μ. The spores on the leaves of the Halsey collection measured 30–45 × 3.0–4.6 μ. Those from both sources were 3-septate.

The spores in the 1945 Granger material sometimes retain the slender, elongate conidiophores. These evanescent appendages could be mistaken for basal cilia on the spores. They arise from compact hyaline to chlorinous mycelial masses. The conidiophore-bearing masses are strictly moniliaceous in nature and are not acervulus-like as in *Pseudodiscosia avenae* Sprague and A. G. Johnson (**14**).

On the basis of present evidence, all of the collections of *Spermospora* on the various genera of grasses belong in the one species, *S. subulata*.

**Phleospora idahoensis** sp. nov.

Maculis nigris, diffusis, pycnidiis immersis, subglobosis v. poculiformibus, gregariis, brunneo-nigris, plerumque leptodermis, parenchymaticis, tarde ostiolatis, 90–150 μ diam.; cirrhis brevibus, hyalinis; pycnosporulis clavato-cylindraceis, rectis v. curvulis, apicibus subacutis, basibus obtusis, 0–1-septatis, hyalinis, 40–53 × 4.6–5.6 μ.

Hab. in foliis et pedicellis vivis *Festucae idahoensis* Elmer, Trude, Idaho, July 28, 1945 (B.P.I. *81,145*, **typus**). Legit George W. Fischer.

Spots black to dark brown, diffuse, pycnidia immersed, subglobose to cup-shaped, brown-black, gregarious, wall thin for the main part (12 μ or less), parenchymatous, finally ostiolate or opening at the top through stomata, 90–150 μ; spore horns short but numerous, hyaline or faintly tinted; pycnospores stoutly club-shaped to somewhat cylindrical, somewhat to distinctly curved, apices tapering, sometimes pointed, bases blunt but the spores narrowed towards the ultimate base, rarely 1-septate, usually aseptate, hyaline with coarse inclusions, 44–53 × 4.6–5.6 μ (FIG. 1, *E*).

There is considerable room for doubt as to whether this fungus belongs in *Phleospora* rather than *Septogloeum* on the one hand or

*Septoria* on the other. Before paraffin sections were made through the fruiting bodies the fungus appeared to belong in *Septogloeum* (**13**) but cross sections of the pycnidia indicate that it does not. The fungus shows strong resemblance to *Septoria infuscans* (Ell. and Ev.) Sprague in the general shape of the spores and pycnidia. The presence of cirrhi is not always typical, the pycnidia are less well developed, have no, or only tardily formed, ostioles, and the spores are wider than the coarsest of the spores of *S. infuscans*. These differences make it seem desirable to place it in a distinct species. Since the pycnidia are ill-formed, it appears to belong in *Phleospora*. This is borne out also by the coarse spores which are more or less typical of *Phleospora*.

*Phleospora idahoensis* is very distinct from *Septogloeum oxysporum* Sacc., Bomm. and Rouss. (**7**). It also is entirely different from *Cylindrosporium calamagrostidis* Ell. and Ev. which has spores 40–60 × 1.5–2.0 μ. Although the spores of *P. idahoensis* have about the same dimensions as those of *P. graminivorum* Sprague and Hardison (**2**) they are very different in shape and in the number of septa. The pycnidia also are different.

*Phleospora idahoensis* derives its name from the specific name of its host and from the state of Idaho where it was found. Fischer collected it a short distance west of the Yellowstone National Park.

### **Ascochyta phleina** sp. nov.

Maculis fulvellis, pycnidiis subgregariis, aureo-brunneis v. brunneis, globosis, erumpentibus, ostiolatis, 100–138 μ; pycnosporulis hyalinis, cylindraceis, utrinque rotundatis, 1-septatis, non constrictis, 11–16 × 1.5–2.2 μ.

Hab. in foliis vivis et dejectis *Phlei pratensis* L. sociis *Helminthosporio* sp., *Cladosporio herbaro* Lk., *Heterosporio phlei* Gregory, *Hendersonia* sp. et *Xanthomonate* sp., prope Lake George, Niawa, Minn., June 4, 1941. **Typus** est B.P.I. *80,920*.

Spots becoming tawny, pycnidia golden brown to brown, globose, strongly erumpent, more or less grouped, ostiolate, 100–138 μ; pycnospores hyaline, cylindrical, both ends rounded with a tendency to be narrowed, contents with few small oil drops, nonconstricted at the one septum, 11–16 × 1.5–2.2 μ, mean size 13 × 1.75 μ, ratio of length to width 7.4 to 1.

This fungus appears to be moderately parasitic. It is sometimes associated with *Heterosporium phlei* and also with what appears

to be *Xanthomonas translucens* var. *phlei-pratensis* Wallin and Reddy on living leaves. In addition it is mingled with several other fungi on necrotic leaves. There had been some late frosts in the area and this contributed to the saprophytic development of some of the forms.

In final analysis this species is recognized because it does not fit any known species of *Ascochyta* on Gramineae. It is too small for the *A. graminicola* Sacc. group. It resembles a fungus on *Distichlis*, which is described in another paper in preparation (**15**), but the spores do not have the cylindrical or capsular shape of that species and in addition are hyaline, not yellow tinted. Morphologically, *A. phleina* may be close to *A. elymi* Tehon and Daniels but the latter species has cylindrical spores, 10–14 × 2–3 μ in pale brown pycnidia (**16**). The writer has seen this obscure species in small quantities a number of times and concludes that the species on *Phleum* is different from it not only because of the thin-walled pycnidia in *A. elymi*, but also because the spores of the latter are broader in the type. *A. phleina* has also been compared with *Septoria phleina* Baudys and Picb. (**1**). This has 3-septate spores similar to *S. triseti* Speg. (**11**). A few multiseptate spores were seen in mounts of *A. phleina* but they were cylindrical and not narrowly obclavate-filiform as in *S. phleina*.

An illustration of *A. phleina* is given in another article which deals exclusively with *Ascochyta* species on western grasses (**15**).

**Phyllosticta healdii** sp. nov.

Maculis diffusis, pallido-brunneis, concoloribus, pycnidiis nigris, numerosis, erumpentibus v. subsuperficialibus, ellipsoideis, plerumque in lineis dispositis, tarde ostiolatis, 111–156 μ longis, 80–115 μ latis; pycnosporulis hyalinis, bacillaribus v. capsuliformibus, aseptatis, medio contractis v. non constrictis, utrinque guttulatis, 11–15 × 3.6–4.5 μ.

Hab. in foliis vivis et emortuis *Panici huachucae* Ashe, Valentine, Nebr. **Typus** est B.P.I. *81,192*. June 15, 1946.

Spots covering most of infected leaves, diffuse, pale brown, of one uniform color, without margin, pycnidia black, more or less grouped in lines, erumpent to nearly superficial, ellipsoidal, mostly definitely longer than broad, 111–156 μ long and 80–115 μ wide, eventually ostiolate, ostiole small; spores in mounts usually need to be crushed from pycnidia, less often emerging through the ostiole; pycnospores hyaline, capsular to bacillar-shaped, non-septate

but many spores having a slight constriction or contraction at the centers; most spores with a prominent inclusion at each end with sometimes one or more smaller inclusions elsewhere, 11–15 × 3.6–4.5 μ (FIG. 1, *F*).

This species is characterized by the numerous black, linearly arranged pycnidia on diffuse brown leaf lesions and the short-cylindric "blocky" spores with their prominent guttulae at each end. The fungus occurs on living leaves and also on dying or dead leaves, some from the previous season. It is apparently perennial on the grass.

There appears to be no species to which this fungus can be referred. *Phyllosticta panici* E. Young (**17**, p. 144) has ovate spores, 4.8–9.6 × 3.6 μ. We have identified *P. panici* on *Panicum virgatum* L. from material collected by Simon Wolff at Guthrie, Okla. and it is different from *P. healdii*. The spores of the latter are very much larger than those of *P. sorghina* Sacc. and are a somewhat different shape from those of *P. rogleri* Sprague on *Digitaria* in Iowa.

The fungus is named for Frederick D. Heald, major professor of the writer during his earlier student years. It was collected in Nebraska, one of the three states in which Dr. Heald founded modern departments of plant pathology.

### **Septoria glycericola** sp. nov.

Maculis prominulis, semiorbicularibus, ochraceis, centro pallidis, pycnidiis aureo-brunneis, subglobosis, ostiolatis, 90–140 μ; pycnophoris papillatis; pycnosporulis lineari-cylindraceis, hyalinis, apicibus subacutis, basibus subobtusis, 1–3-septatis, 20–34 × 1.9–3.4 μ.

Hab. in foliis et vaginis vivis *Glyceriae striatae* (Lam.) Hitchc. prope Hoover, Oreg. (**typus** est O.S.C. *10,177*). Coll. H. P. Barss and G. B. Posey. Paratypes: *Glyceria striata*, White Hall, Ky. (J. H. Hardison); *G. canadensis* (Michx.) Trin., Dennis, Mass. (C. L. Lefebvre); *G. elata* (Nash) Hitchc., near Elgin, Oreg. (B.P.I. *80,113*, G. W. Fischer); *G. grandis* S. Wats., Bismarck, N. Dak. (Laird Wolff, B.P.I. *81,188*); *G. pauciflora* Presl, Cascade Mts., Wash. (Sprague); ibid., Lawyer Canyon, Idaho (Sprague, Fischer, Meiners, C.S. *3679*).

Spots prominent, buff, semiorbicular, becoming paler in the center, pycnidia scattered, golden brown, subglobose, slightly flattened, walls 6–7 μ thick, composed of rectangular cells which finally collapse to a corky parenchymatous structure, pycnidia

90–140 μ diam., up to 90 μ tall; pycnophores absent, obscure, or short papillae, pycnospores narrowly cylindrical, somewhat pointed to blunt at apex, rounded at the base and usually definitely blunter than at apex, hyaline, 1–3-septate, 20–34 × 1.9–3.4 μ, mean size about 25–26 × 2.8 μ (FIG. 1, *G*).

This fungus was originally assigned to *Stagonospora glyceriae* Roum. and Fautr. but after studying more and better material it was very clear that it was not that species. Later (**13**) the fungus was placed tentatively in *Septoria nodorum* Berk. but with a notation that it might need to be classed as a distinct species. In studying a great mass of material of *S. nodorum* on a considerable number of hosts, the collections from *Glyceria* always constituted a distinct group because of their proportionately narrower spores with the pointed ends. The writer concluded that this was a case where his efforts to reduce the number of species of *Septoria* on grasses would only lead to confusion, and he has therefore followed the earlier suggestion of Lefebvre (personal letter) to describe the fungus as a new species.

Material collected July 10, 1946 at Bismarck, N. Dak., on *G. grandis* appears to be a summer stage with small spores in the pycnidia. The pycnidia, which are in faded spots, are minute (60–80 μ), black, the spores 0–1-septate, 10–15 × 1.3–1.6 μ, but have the characteristic pointed apex.

The material from Kentucky, sent by Hardison, has some of the spores blunter at the ends (FIG. 1, *H*) and hence closer to *S. nodorum* in shape but nevertheless still somewhat narrowed for that species.

### SEPTORIA PASSERINII Sacc. on HYSTRIX PATULA Moench

*Septoria microspora* Ellis, with spores 6–12 × 0.7–1.2 μ, was elsewhere referred to *S. passerinii* (**11**), being considered a microspore stage of that species. In 1945, material of *Septoria* spp. on *Hystrix patula* was collected at Cotton Lake, Minn. (B.P.I. *81,163*). Most of the material has microspores 3–5 × 0.5–0.8 μ and macrospores 55–64 × 1.8–2.4 μ (FIG. 1, *I*) borne in black, carbonaceous, erumpent, globose pycnidia, 120–205 μ in diameter. The macrospores are filiform, somewhat pointed at the apex, less

pointed at the base. They are faintly 3-septate, hyaline with small hyaline inclusions. The spores are proportionately longer than the general concept of the stubby spored *S. passerinii,* which usually has macrospores 22–45 × 1.5–2.2 μ. However as reported earlier (11), overwintering material on *Hordeum nodosum* L. has spores as large as 56 × 2.7 μ. We saw somewhat similar material on *Hordeum distichon* L. at Sundance, Wyo., in June, 1946. It appears conservative therefore to include the *Hystrix* fungus in *S. passerinii.*

In additon to *S. passerinii,* the collections from Cotton Lake contain a smaller amount of a species of *Septoria* with 3-septate spores, 29–33 × 2.5–3.1 μ. These spores (FIG. 2, *A*) are cylindrical, very distinct from *S. passerinii* and somewhat intermediate in size between *S. nodorum* Berk. and *S. avenae* Frank. The fungus may be similar to a species on wheat, *Elymus* spp., and *Agropyron* spp. in the western United States and Eurasia which has not been satisfactorily classified as yet. The fungus may belong in this group, which is typified by *S. avenae.* However, it seems to the writer that it is somewhat less robust than *S. avenae* and can better be placed in *S. nodorum* at this time. In this connection, a common, purple to fuscous blotch on the marsh grass *Fluminea festucacea* (Willd.) Hitchc. appears to belong in *Septoria avenae* (FIG. 2, *B*). The writer had assigned this to his polymorphic concept of *Stagonospora arenaria* Sacc. but the spores in a majority of the collections from North Dakota and South Dakota are close to *S. avenae,* averaging about 27–33 × 2.4–3.0 μ. Most of them are 1-septate summer spores. Therefore, although they average about the same size as those of the fungus on *Hystrix,* the different environment indicates that they are likely to prove larger than that under later summer conditions. The species of this entire group, ranging from the small *S. nodorum* to the somewhat larger *S. avenae* to the similar but still coarser *St. arenaria,* are difficult to separate in many cases. There are strong indications that all three, if they are all distinct, are not confined to any particular group of hosts, although *St. arenaria* appears to be commonest on *Elymus* and *Agropyron, S. avenae* on oats and oat relatives, and *S. nodorum* occurs on many grasses. One solution to this problem may be through a detailed study of the ascigerous stage of *S. avenae*

(*Leptosphaeria avenaria* G. F. Weber), which may occur on other hosts than oats.[3]

SEPTORIA QUINQUESEPTATA Sprague on KOELERIA CRISTATA (L.) Pers.

*Septoria quinqueseptata* was obtained on *Koeleria cristata* in the plots at Mandan, N. Dak., on Aug. 28, 1943 (B.P.I. *80,871*). At that time the spores were obscurely 3-septate, 38–55 × 2.2–2.7 μ. Two years later when re-examined, they had become very clearly 5–7-septate, mostly 5-septate. It is not unusual to find that viable herbarium material continues to develop for a time after collecting and before desiccation checks further changes. In 1943, the spores in pure culture on potato-dextrose agar were 38–55 × 1.9–2.4 μ (FIG. 2, *C, a*). The colonies were cottony to sub-cottony and were rosy buff in color.

*Septoria quinqueseptata* on *Koeleria cristata* differs from the common *Septoria calamagrostidis* f. *koeleriae* (Cocc. and Mor.) Sprague in several ways. The spores of the latter are much narrower, 35–82 × 1.1–2.1 μ (FIGS. 2, *C, a* and *b*; 2, *D*). This species also produces a mucose or wet yeasty growth in pure culture, with rosy buff cottony mycelium formed only after the colonies begin to stale in old cultures (**11**).

A comparison of the Mandan material on *K. cristata* with the type of *Septoria quinqueseptata* on *Sphenopholis obtusata* (Michx.) Scribn. (**11**) convinces the writer that it belongs in that species. The spores of this fungus in both the type and on *K. cristata* are too narrow and too short for *S. andropogonis* f. *sporobolicola* Sprague (**9**, FIG. 1, *A*).

Some confusion existed in the determination of the host of the Mandan material. *Koeleria cristata* was growing intermixed with the rhizomatous species of grass, *Calamagrostis montanensis* Scribn., which was rapidly crowding out *K. cristata*. As the two species are superficially similar, careful check was later made to determine if the host was not actually *Calamagrostis*. It was de-

[3] Since our paper went to the printer a long anticipated, enlightening article by T. Johnson has appeared: A form of *Leptosphaeria avenaria* in Canada. Canad. Journ. of Research **25** (*6*) : 259–270.

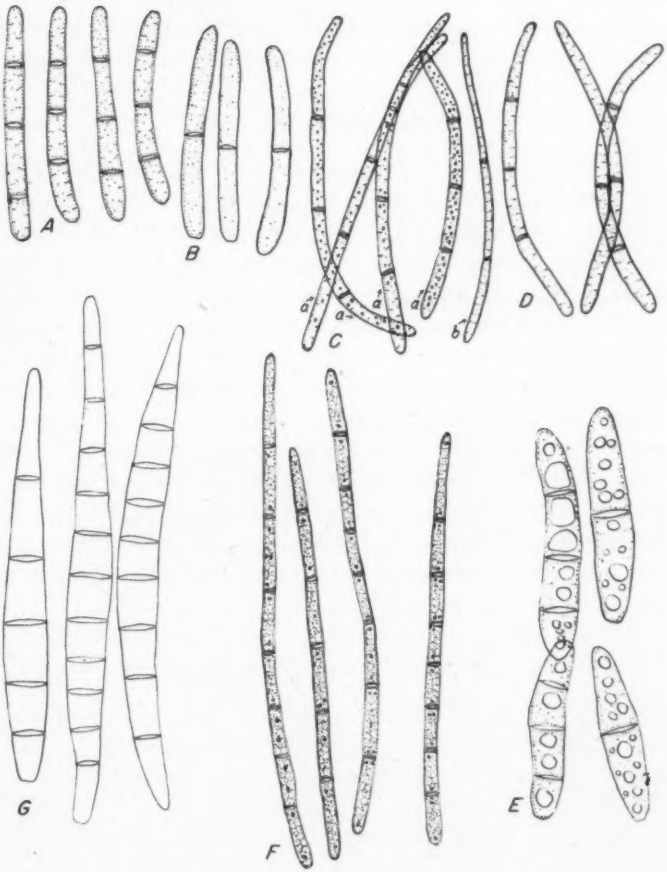


FIG. 2. Spores of leaf spot fungi on western grasses.

termined beyond a doubt from fruiting material of the host, however, that the fungus in question was growing on *K. cristata.*

*Septoria quinqueseptata* is doubtfully parasitic on *Koeleria cristata* and possibly represents an accidental development on this grass. Apparently it does not occur on this host at all commonly.

### SEPTORIA TANDILENSIS Speg.

This species, which was discussed recently (**12**, p. 54), has since been collected on *Panicum capillare* L. at Mandan, N. Dak., August 13, 1946. The collection was made a few hours after a hard rain which broke a long drought. The spores, which were typical otherwise, had evidently taken up moisture and started to germinate *in situ.* Some were swollen and as large as 111 × 2.7 μ, others were normal size. The latter were 50–80 × 1.3–1.6 μ with all gradations between that size and the swollen ones. The curved, hyaline, faintly multiseptate spores, except in swollen ones, were borne in typical black pycnidia in small black, drought-inhibited spots. In spite of the aberrant aspect of the spores, this fungus is unquestionably *S. tandilensis.* This is the first report of it in the plains area of North Dakota and represents nearly a thousand miles extension of its range westward from Wisconsin, where it is well known.

### SEPTORIA CENCHRINA J. J. Davis

This species was collected on *Cenchrus pauciflorus* Benth. at Mandan, North Dakota, Aug. 14, 1946, an extension of its range from Minnesota into the plains country. It produced pale obscure pycnidia on yellowed leaves without appreciable spot formation although some lesions were delimited linearly by the leaf veins. No doubt the fungus is commoner than collections show because the symptoms are very obscure.

Pure cultures were readily obtained on potato dextrose agar. The light-cream colored juvenile colonies developed, at room temperature, into black mounded carbonaceous growths from which minute spore masses exuded. The spore masses did not run together but remained separate as flesh colored to dirty pink pin

points over the black carbonaceous mass of the culture. The cultures were more vigorous than those of *S. infuscans* but tended towards a similar type of growth rather than like the mucose colonies produced by most species of *Septoria* on grasses. The spores in pure culture were typically long and straight-sided without appreciable difference in dimension from one end to the other. They were faintly 3–7-, mostly 5–7-septate under the most critical illumination. This is interesting because it had not been determined just how many septa, if any, spores of this species had. The septa were readily seen with the N. A. 1.32 lens. The contents were opaque under critical illumination but with a lens with a longer focal distance, N. A. 1.15, which the writer frequently uses, the spores appeared faintly yellowish. Some of them were as large as 125 × 2.4 μ.

**Stagonospora glycericola** sp. nov.

Maculis nullis v. stramineis, pycnidiis globosis, erumpentibus, non gregariis, aureo-brunneis, 90–140 μ diam.; pycnosporulis subcylindraceis, v. subfusiformibus, constrictis v. non constrictis, apicibus et basibus subobtusis, hyalinis, globulatis, 1–3-septatis, 22–30 × 4.6–6.4 μ.

Hab. in foliis languidis *Glyceriae striatae* (Lam.) Hitchc., Niawa, Minn. **Typus** est B.P.I. *81,180* et paratypus in *G. grandis*, Edinburg, N. Dak. (B.P.I. *81,189*).

Spots none to straw colored, pycnidia globose, erumpent, nongregarious, golden-brown, 90–140 μ diam.; pycnospores subcylindrical to subfusiform, constricted to non constricted, apices and bases subobtuse, hyaline, globulate, 1–3-septate, 22–30 × 4.6–6.4 μ.

On sickly leaves of *Glyceria striata* (Lam.) Hitchc., Minn.

This fungus (FIG. 2, *E*) might be referable to *St. subseriata* (Desm.) Sacc. except that the spores of the latter are so much more fusiform or even boat-shaped (**8**, FIG. 1, *B, D*). In shape it is possibly closer to *St. subseriata* var. *maculata* Grove (**8**, FIG. 1, *A*) but this active parasite of *Dactylis glomerata* L. differs in many ways from the fungus on *Glyceria*. Incidentally, the writer considers that *St. subseriata* var. *maculata* Grove deserves specific rank, and accordingly **Stagonospora maculata** (Grove) comb. nov. is proposed. This fungus is becoming recognized as the cause of an important leaf disease of orchard grass, especially in pastures in the Middle Atlantic states.

A search of available species of *Stagonospora* does not disclose any on grasses that are similar enough to *St. glycericola* to justify assigning the two collections on *Glyceria* to a previously described species. According to description alone, *St. glyceriae* Roum. and Fautr. is closer to *Ascochyta graminicola* var. *brachypodii* Trail than to our fungus. The 1-septate spores of *S. glyceriae* are $16 \times 4\ \mu$. Those of *A. graminicola* var. *brachypodii* are $22–25 \times 5.6–6.2\ \mu$. Thus the evidence is against *St. glyceriae* being a phase of *St. glycericola*, and so they are considered distinct. Another species, *St. glyceriae* Kupka (**4**, p. 162), is even more distinct from *St. glycericola*. *St. glyceriae* Kupka has non-hyaline spores, $50–80 \times 3–4\ \mu$, with 7–11 septa, all of which means that it probably belongs in the genus *Phaeosceptoria*, probably *P. festucae* Sprague (**10**), which has spores $50–85 \times 2.8–4.8\ \mu$ with 8–11 septa. Incidentally we have collected *P. festucae* on *Glyceria grandis* in North Dakota although another collection (B.P.I. *80,072*) from Pingree, N. Dak., was finally assigned to *Hendersonia crastophila* Sacc. because the spores were too short for *P. festucae* Sprague.

In considering graminicolous species of *Stagonospora* not on *Glyceria*, except for *St. subseriata*, there are few species of this genus in the 3-septate group with spores within the size range of *S. glycericola*. One of these is *S. alopecuri* Rostr. It has spores $25–32 \times 5–6\ \mu$ but the pycnidia are listed as gregarious and prominent and the spores are bacillar-cylindrical, none of these characters being at all like those of *St. glycericola*. *Stagonospora brachypodii* Died. answers the description of *S. glycericola* very well except that the darker pycnidia and yellow spores indicate, as Saccardo says (**5**, v. 25, p. 367), that the fungus is close to *Hendersonia culmicola f. minor* Sacc., which is different from ours.

**Phaeoseptoria poae** sp. nov.

Pycnidiis nigris, carbonaceis, globosis, subsuperficialibus, $150–220\ \mu$, ostiolatis; pycnosporulis chlorinis, filiformi-clavulatis, apicibus acutis, $50–77 \times 2.1–2.4\ \mu$, 5–7-septatis.

Hab. in foliis emortuis *Poae canbyi* (Scribn.) Piper (*P. lucidae* Vasey). Custer National Forest, prope Ekalaka, Mont., June 21, 1945 (B.P.I. *81,144*).

Pycnidia black, fragile or brittle, carbonaceous, globose, subsuperficial, 150–220 $\mu$, ostiolate; pycnospores yellow, filiform-clavulate, pointed at the apex, slightly blunted at the base, 50–77 $\times$ 2.1–2.4 $\mu$, 5–7-septate (FIG. 2, *F*).

Habitat in dead leaves of *Poa canbyi* in the hills in open scrub woods, Custer National Forest near Ekalaka, Mont.

*Phaeoseptoria poae* differs from *P. airae* (Grove) Sprague in having nearly filiform spores with 5–7 septa, whereas *P. airae* has wider spores with 9–10 or even 13 septa (**10**, FIG. 1, *G*).

The host in the type material of *P. poae* appears to be a pale green form of *Poa canbyi* sometimes known as *Poa lucida* Vasey, which Hitchcock reduces to synonymy (**3**).

A collection of *Phaeoseptoria festucae* was made on *Poa nevadensis* Vasey at Mandan, N. Dak. (B.P.I. *80,866*). This species has distinctly broader spores than *P. poae*. *P. festucae* appears to be widespread but a minor saprophyte on a number of grasses. It has been collected on *Festuca rubra* and *Danthonia parryi* Scribn. as well as on *Glyceria grandis* mentioned earlier in the article. A form of it has been found on *Muhlenbergia mexicana* (L.) Trin. (**10**). This same form occurs on *Elymus virginicus* L. in North Dakota. A form on *Andropogon furcatus* Muhl. with very coarse spores (FIG. 2, *G*) is being described separately as a new variety of *P. festucae*.[4]

The writer wishes to thank Dr. A. G. Johnson, Miss Edith K. Cash and Mr. John A. Stevenson for aid in the preparation of this and earlier papers. The mycological notes issued by the writer from time to time during the past 18 years have been a by-product of more pressing studies on diseases of Gramineae. They have been offered because there was need for even incomplete data, such as these, on the leaf spots of cereals and grasses. There is a great deal to be done in the way of critical life history studies in this group.

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[4] The description will be included in a paper by H. C. Greene, the fungus having been collected by him in Wisconsin and sent to the writer for determination.

## EXPLANATION OF FIGURES

FIG. 1. *A*, conidia of *Spermospora subulata* on *Festuca rubra*, Granger, Ore., 1945; *B*, conidia of *S. subulata* on *Deschampsia caespitosa*, Beartooth Pass, Mont.; *C*, conidia, macrospores, and microspores, of *S. subulata* in pure culture on potato dextrose agar from *Festuca rubra*, west of Halsey, Ore., 1937; *D*, conidia of *S. subulata* on *F. rubra*, west of Halsey, Ore., 1937; *E*, pycnospores of *Phleospora idahoensis*, type; *F*, pycnospores of *Phyllosticta healdii* on *Panicum huachucae*, ex type; *G*, pycnospores of *Septoria glycericola* on *Glyceria canadensis*, Dennis, Mass.; *H*, pycnospores of *S. glycericola* on *Glyceria striata*, White Hall, Ky.; *I*, pycnospores of *S. passerinii* on *Hystrix patula*, Cotton Lake, Minn. (all × 1000).

FIG. 2. *A*, Pycnospores of *Septoria nodorum* on *Hystrix patula*, Cotton Lake, Minn.; *B*, pycnospores of *Septoria avenae* Frank on *Fluminea festucacea*, Sand Lake, S. Dak.; *C*, *a* pycnospores of *Septoria quinqueseptata* from pure culture (potato-dextrose agar) isolated from *Koeleria cristata*, Mandan, N. Dak., compared with *b* pycnospore of *S. calamagrostidis* f. *koeleriae* from Corvallis, Ore.; *D*, pycnospores of *Septoria quinqueseptata* on *Koeleria cristata*, Mandan, N. Dak.; *E*, pycnospores of *Stagonospora glycericola*, ex type; *F*, pycnospores of *Phaeoseptoria poae*, ex type; *G*, pycnospores of *P. festucae* var. ined. (all × 1000).

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# HYPHAL PROLIFERATION THROUGH CLAMP-FORMATION IN POLYPORUS CINNABARINUS FR.[1]

JOHN B. ROUTIEN [2]

(WITH 1 FIGURE)

Though Kniep (**3**, figs. 9 and 10 and **4**, fig. 2) and Bensaude (**1**, fig. 18) long ago illustrated in certain Agarics the origin of some branches of the hyphae from clamp-connections, this behavior appears to be mentioned rarely in mycological literature. Drechsler (**2**) also has illustrated the development of branches from clamp-connections in *Nematoctonus haptocladus,* a fungus parasitic on nematodes. In 1936 Rogers (**5**) described in a new *Sebacina* a process of repeated branching of the clamp-connections basal to the basidia. He stated (**5**), "The binucleate apical cells . . . develop into basidia, the second basidium being borne on the clamp at the base of the first. By repetition of this process a unilaterally cymose cluster of basidia is formed."

Casual examination of the hyphae of certain tissue-cultures of sporophores of *Polyporus cinnabarinus* Fr. revealed that many branches developed from clamp-connections, and it seemed desirable to describe the formation of these branches in detail.

Tissue-cultures from two sporophores growing on the same log, but about 15 ft. apart, showed abundant branching from the clamp-connections. Another culture from a sporophore collected about one-fourth mile from the afore-mentioned specimens, however, showed no branches arising from clamps.

Twenty single-basidiospore cultures derived from one of the first-mentioned sporophores were paired in all possible combinations and examined microscopically. Branches developing from

[1] Contribution from the Mycology Section, Chas. Pfizer & Co., Brooklyn, N. Y.

[2] The author acknowledges gratefully the technical assistance of Miss Dorothy E. Meyn.

clamp-connections were seen in nearly all of the dicaryon cultures that developed from these matings. Presumably, closer examination would have revealed their presence in all pairings.

To determine the nuclear behavior in these branches arising from clamp-connections, transfers of one of the tissue cultures were grown on a small amount of potato-dextrose-agar on a sterile slide in a damp chamber at 28° C. for 3–4 days. The specimens then were killed and fixed in strong chrom-acetic acid mixture and stained with Harris' Hematoxylin (**6**). Nuclear figures of the stained slides are interpreted in the following manner.

The dicaryon condition of the cells of the culture is maintained through the generation of clamp-connections accompanying conjugate nuclear division. Branches may develop near the terminal end of a cell at some point other than from a clamp-connection, but apparently a clamp-connection develops very early in the ontogeny of such a branch in the culture used.

More frequently, however, in this particular culture, a branch will arise from a very young (FIG. 1, *a*) or moderately older clamp-connection and will grow out at an acute or obtuse angle to the main hypha. At first the nuclei of the cell subtending the clamp-connection are in about the middle of the cell (FIG. 1, *b*), but very soon after initiation of the branch they may be found near the clamp-connection (FIG. 1, *a*). A little later one of the nuclei will be seen in the branch with the second nucleus apparently about to enter (FIG. 1, *c* and *d*). When the branch is only slightly longer two nuclei may be seen in it, whereas the cell subtending the clamp-connection shows no evidence of nuclear material (FIG. 1, *e*). The next stage found is that in which the two nuclei are undergoing conjugate nuclear division (FIG. 1, *f*). This is followed by the formation of a short branch directed away from the tip of the cell with one of the daughter nuclei in its tip, one in the cell of the main hypha and two in the branch (FIG. 1, *g*). This short branch can be seen to fuse with the cell of the main hypha (FIG. 1, *h*) at about the time that septa delimit the new dicaryon cell from the original cell (FIG. 1, *h*). Sometimes, however, these septa do not form so soon (FIG. 1, *i*). The final condition, then, can be seen in figure 1, *j*.

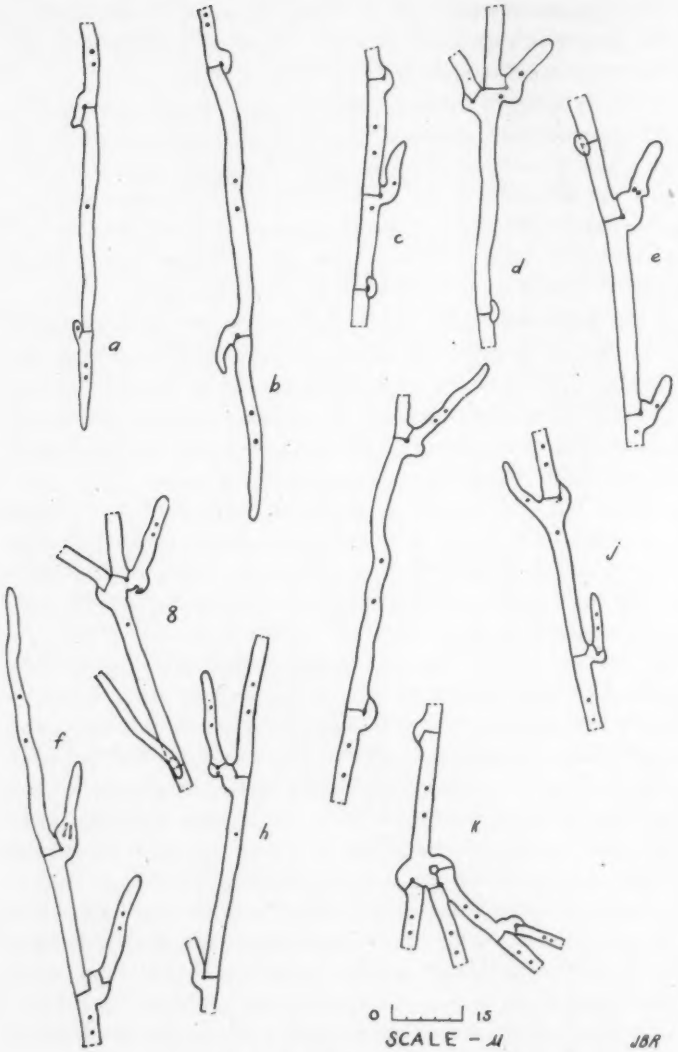


FIG. 1. Development of hyphae from clamp-connections in *Polyporus cinnabarinus*.

Much more complex figures are not uncommonly found; figure 1, *k* represents such a system. This particular figure can be explained as follows: opposite the clamp-connection on the main hypha a branch began to develop that immediately formed a large clamp-connection. From the surface of this clamp-connection another branch and clamp-connection began to develop simultaneously. Septa cutting off the clamp-connections from the cells are oriented peculiarly because the two clamp-connections are so near each other.

The nuclear behavior described here seems to be the same as that described by Kniep (**3**) for *Corticium varians* and (**4**) for *Collybia conigena,* by Bensaude (**1**) for *Tricholoma nudum* and by Rogers (**5**) (for basidial proliferation) in a *Sebacina.*

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## EXPLANATION OF FIGURES

FIG. 1. *Polyporus cinnabarinus* Fr. Free-hand drawings made from preparation examined at approximately 1200 magnification with compensating eye-pieces. Only nuclear contents of cells are shown. *a.* Terminal portion of a hypha showing very young branch developing from a clamp-connection with nuclei of ante-penultimate cell near the clamp-connection; also shown is a nearly complete clamp-connection in the terminal cell. *b.* Slightly longer branch developing from clamp-connection; nuclei involved still are in the middle of the cell. *c.* Short branch from a clamp-connection; one nucleus has entered the branch, while the second is about to enter. *d.* Slightly more

advanced stage. *e*. Stage in branch development in which both nuclei have entered the branch; note start of formation of new clamp-connection. *f*. Conjugate nuclear division of the two nuclei in the branch. *g*. Further development of clamp-connection. *h*. New clamp-connection is completely formed; new branch has two nuclei, and parent cell still is dicaryon. *i*. New branch is formed with two nuclei and parent cell has two nuclei; septa separating the two "cells" have not formed. *j*. Final stage in which both nuclei of the parent cell have returned to about the middle of the cell. *k*. Structure produced by development of branches and clamps in rapid succession.

# THE BOLETES OF YUNNAN [1]

WEI FAN CHIU [2]

In the latter part of the nineteenth century foreign explorers such as Potanin and others who collected higher plants in this country incidentally picked up fungi at the same time (Tai: Sci. Rept. Nat. Tsing Hua Univ. Ser. B **2**: 137–139, 1936). Of their fungus collections two new genera and ten species (including three new) of the Boletaceae were reported (Patouillard: Bul. Soc. Myc. Fr. **11**: 106, 1895; Kalchbrenner: Bul. Acad. Imp. Sci. St. Peterb. **27**: 135, 1881; Lohwag: Simbolae Sinicae **2**: 54, 1937). Since 1932, Teng carried on a study of the fleshy fungi of China, and recorded, up to 1938, twenty-three species of this group, including one new species (A Contr. to Our Knowledge of the Higher Fungi of China. Acad. Sinica, 1939). In the present paper fifty-four species and two varieties, including twenty-two new species and one new variety, are recorded. All of the specimens, except a few, were collected in Yunnan in the Southwest of China.

## BOLETINUS Kalchbrenner

**Boletinus kunmingensis** sp. nov.

Pileo 3–5 cm. lato, pulvinato dein convexo, "Baryta Yellow," [3] aetate "Tawny," viscido, glabro; tubulis 3–4 mm. longis, "Pinard Yellow" v. "Buff Yellow," decurrentibus; poris majusculis, plus minus hexagonalibus v. alveolatis, radiatim dispositis; stipite 3–4 cm. longo, 3–7 mm. crasso, "Baryta yellow," subaequali v. deorsum attenuato, fusco-punctato; carne flava. Sporis pallide olivaceis, 9–11 × 4–5 μ (10 × 5 μ).

[1] Paper No. 17 from the Department of Plant Pathology, College of Agriculture (formerly Institute of Agricultural Research), National Tsing Hua University, Peiping, China.

[2] This work was done under the direction of Prof. F. L. Tai to whom the writer wishes to express his indebtedness for encouragement, valuable suggestions and help in the preparation of the manuscript. Thanks are also due to Dr. W. H. Snell of Brown University for reading the manuscript and making valuable suggestions.

[3] Refer to Ridgway, R. Color Standards and Color Nomenclature. Washington, D. C., 1912.

Pileus 3–5 cm. in diameter, at first pulvinate to hemispherical, becoming convex, "Baryta Yellow" when young, "Tawny" with age, very viscid when moist, glabrous; margin wavy. Tubes 3–4 mm. long, short-decurrent, "Pinard Yellow" or "Buff Yellow"; pores large, hexagonal, radially arranged, dotted with glandules. Stipe 3–4 cm. long, 3–7 mm. thick, "Baryta Yellow," subequal or tapering downward, dotted with dark-colored granules, stuffed. Flesh yellowish, paler in the cap.

Spores pale olivaceous under the microscope, elliptical, 9–11 × 4–5 $\mu$ (10 × 5 $\mu$).

Nov. 12, 1941, W. F. Chiu, Tiehfungan, Kunming (*7695*), **Type.**

This fungus is close to *B. punctatipes* Snell et Dick, but differs from the latter in its larger spores. It has the same habitat as *B. punctatipes* Snell et Dick var. *pinetorum,* but it differs from the latter in the entirely punctate and yellow-colored stipe.

BOLETINUS PUNCTATIPES Snell et Dick var. **pinetorum** var. nov.

A typo differt stipite semper subconcolore nunquam albido, apice non reticulato.

Pileus 4–9 cm. in diameter, hemispherical, becoming broad convex to plane, "Flesh Ochre," "Cinnamon Rufous" or "Orange Cinnamon," very viscid when moist, glabrous; margin wavy. Tubes 3–5 mm. long, light "Cadmium Yellow," decurrent; pores large, 3–4 × 1–2 mm., compound, radially arranged, concolorous with the tubes, dotted with glandules. Stipe 2–5 cm. long, 5–15 mm. thick, subconcolorous with the cap but usually paler, yellowish at the apex, minutely punctate at the upper part of the stipe with brown dots, rarely slightly reticulate at the apex, tapering downward. Flesh white or pinkish in the cap, yellowish adjacent to the tubes and in the stipe, "Orange Yellow" at the basal part of the stipe.

Spores pale olivaceous under the microscope, elliptical, 6–11 × 3–4 $\mu$ (8 × 3 $\mu$).

June to Aug. 1940, F. L. Tai and C. C. Cheo, Tapugi, Kunming (*7888* and *7725*); Tiehfungan, Kunming, Nov. 12, 1941, W. F. Chiu (*7717* **Type**, *7720* and *7730*); Aug. 1938, C. C. Cheo, Tali (*7691*).

This variety differs from var. *typica* in that its stipe is never white or whitish but instead concolorous with the cap and that

the reticulation at the apex of the stipe is rare to occasional. The American plant occurs commonly under firs whereas the Yunnan form is commonly associated with pines (*Pinus yunnanensis*).

BOLETINUS PICTUS Peck

Pileus 2–13 cm. in diameter, hemispherical, convex becoming plane, dry, "Pompeian Red" or "Morocco Red," at first paler, then darker, covered with adpressed fibrils and scales; scales separated in later development, and becoming "Brussels Brown"; "Light Cadmium" yellow between and beneath the scales. Tubes 3–5 mm. long, "Light Cadmium" yellow, decurrent; pores 1–2 mm. across, concolorous with the tubes, becoming ochraceous with age, radially arranged, angular, compound. Stipe 6–12 cm. long, 8–35 mm. thick, yellow above the ring, "Light Buff" or "Warm Buff" below, tinged "Morocco Red" in the lower portion, coarsely reticulate or streaked, sometimes fibrous, turning reddish-brown with age, subequal or ventricose at the base; ring grayish, membranous or rather cottony. Flesh "Pale Yellow Orange," pinkish in the stipe.

Spores olivaceous under the microscope, elliptical, 9–11 × 3–4 μ (9 × 4 μ).

Aug. 24, 1938, C. C. Cheo, Chunghotze, Tali (*7782, 7783* and *7781*); July 23, 1938, C. C. Cheo, Chenkung market (*7890*); Aug. 12, 1942, W. F. Chiu, Shishan, Kunming (*7880* and *7881*). Usually under *Pinus Armandii*.

The typical forms of this species were collected under pines in the vicinity of Kunming, but variation was noted in that the squamules of the cap usually change to "Brussels Brown" in the later stage of development, or are of such a color even when young. The stipe is not always cylindrical but sometimes bulbous. The fibrillose and adpressed scales may be also present on the stipe in certain cases.

BOLETUS Fr.

**Boletus minutus** sp. nov.

Pileo 1–3 mm. lato, carnoso, pulvinato dein convexo, glabro, "Sudan Brown"; tubulis usque ad 0.5 mm. longis, ochraceis, decurrentibus; poris imperfectis, daedaloideis, griseo-brunneis; stipite 3–4 mm. longo, 0.6–1 mm. crasso, subaequali, leviter ventricoso v. sursum attenuato, pileo concolore, glabro, farcto; carne brunneola, immutabili; sporis olivaceis, ellipsoideis, 4.5–7 × 2–2.5 μ (6 × 2.5 μ).

Pileus 1–3 mm. in diameter, pulvinate to convex, glabrous, "Sudan Brown." Tubes up to 0.5 mm. long, ochraceous, short-decurrent; pores daedaloid, 0.1–0.2 mm. across, grayish brown. Stipe 3–4 mm. long, 0.6–1 mm. thick, slightly ventricose or tapering upward, concolorous with the cap but paler, glabrous, stuffed. Flesh brownish, unchanging.

Spores olivaceous under the microscope, elliptical, 4.5–7 × 2–2.5 μ (6 × 2.4 μ).

June 25, 1942, Miaokaotze, Kunming, W. F. Chiu (*8041*), **Type.** On mossy slope in mixed woods.

This plant is outstanding for its minute stature. Its hymenophore, as far as observed under the microscope, does not uniformly consist of tubes with round pores. Some tubes possess a somewhat daedaloid orifice. Furthermore, its typical small spores are also rare in boletes. At present, it is tentatively placed in the genus *Boletus*.

BOLETUS CASTANEUS Fr. ex Bull.

Pileus 4–6 cm. in diameter, convex to plane, "Russet Brown" to "Amber Brown," minutely tomentose. Tubes short, about 2.5–3 mm. long, at first white then yellowish, free from the stipe; pores less than 1 mm. across, whitish. Stipe 5–8 cm. long, 1–2 cm. thick, concolorous or paler than the cap, minutely tomentose, subequal, stuffed. Flesh white, unchanging.

Spores hyaline or subhyaline under the microscope, short ellipsoid, 8–9 × 5–6 μ (8 × 5.5 μ), rarely reaching 10 μ long.

July 13, 1938, C. C. Cheo, Chunchutze, Kunming (*7884*); Aug. 28, 1938, C. C. Cheo, Tali (*7702* and *7806*).

BOLETUS FLAVIDUS Fr.

Pileus 3–7.5 cm. in diameter, semiglobose to convex, glabrous, "Sayal Brown." Tubes up to 4 mm. long, white; pores small, roundish, free. Stipe 2–3.5 cm. long, 1–1.5 cm. thick, concolorous with the cap, glabrous, subventricose. Flesh white, unchanging.

Spores hyaline under the microscope, oblong-elliptical, 3.5–5 × 5–7.5 μ.

July 27, 1939, W. F. Chiu, Mt. Omei, Szechwan (Chiu *135*).[4]

[4] Specimens collected outside Yunnan and deposited in the Herbarium of the Plant Pathology Department, University of Nanking, Nanking, China.

**Boletus atroviolaceus** (v. Höhn.) comb. nov. (*Suillus atrovio-laceus* v. Höhn. Frag. zur Myc. n. 835 in Sitzgsb. Akad. Wiss. Wien CXXIII (1): 87, 1914).

Pileus 2.6 cm. in diameter, plane to convex, "Bluish Violet," tinged "Tawny" at the margin, minutely tomentose. Tubes short, 3 mm. long, white, free from the stipe; pores 0.2 mm. across, white, turning reddish-brown with age, angular, simple. Stipe 3.5 cm. long, 4–9 mm. thick, concolorous with the cap above, "Tawny" below, granulose-tomentose in the upper portion, subventricose in the lower part, hollow. Flesh whitish.

Spores hyaline under the microscope, short-ellipsoid, 8–11 × 6–8 μ (9 × 6 μ).

July 16, 1938, C. C. Cheo, Shishan, Kunming (*7901*).

BOLETUS FLAVUS With.

Pileus 2–6 cm. in diameter, convex, very viscid when moist, shining when dry, "Pinard Yellow" to "Empire Yellow," slightly rugose with age. Tubes up to 6 mm. long, "Aniline Yellow," becoming "Orange Citrine," compound, adnate decurrent; pores up to 2 mm. across, somewhat smaller near the marginal region, concolorous with the tubes, angular, compound. Stipe 4–7 cm. long, 6–12 mm. thick, whitish to yellowish or brownish at the base, punctate with dark-brown elements, subequal, stuffed then hollow, slightly reticulate at the apex; ring whitish, thin-membranous. Flesh yellowish, unchanging.

Spores olivaceous under the microscope, elliptical, 8–11 × 3.5–4.5 μ (8.5 × 3.5 μ).

Aug. 11, 1942, W. F. Chiu, Shishan, Kunming (*7849* and *7858*). Under *Pinus Armandii*.

The present fungus agrees closely with the European form except that the dotted elements on the stipe may extend downward below the ring.

BOLETUS LUTEUS Fr. ex L.

Pileus 3.5–5 cm. in diameter, convex to plano-convex, "Cinnamon Buff" to "Tawny," viscid when young and moist, becoming glabrous and shining when dry, finely virgate with age. Tubes 3–4 mm. long, yellow, becoming olive, short-decurrent; pores minute, 2–3 per mm., yellow. Stipe 2.5–4 cm. long, 5–10 mm. thick, yellowish, reticulate on the upper part of the stem from the decurrent walls of the pores, punctate with brownish elements above

and below the ring; ring thin-membranous, brownish and tinged with purplish; veil becoming shredded but sheathing the lower part of the stipe when young. Flesh firm, whitish or yellowish.

Spores olivaceous under the microscope, elliptical, spindle-elliptical, 8–9 × 3–3.5 μ (8 × 3 μ).

Aug. 24, 1940, F. L. Tai, Kunming (*5573*); Aug. 5, 1942; Tapugi, W. F. Chiu (*7856*).

The spores of the Yunnan form are very close to those described by Singer (Florida Boletineae II: 271, 1945).

BOLETUS THIBETANUS Pat.

Pileus 2–3.5 cm. in diameter, pulvinate to convex, "Liver Brown," pale toward the margin, reticulate in the central portion, very viscid when moist, densely covered with dark-brown-dotted elements when young; margin often appendiculate with gelatinous veils. Tubes 4–9 mm. long, "Lemon Chrome" or "Chalcedony Yellow," adnate-uncinate; pores up to 1 mm. across, concolorous with the tubes, angular, simple. Stipe 3–6 cm. long, 3–6 mm. thick, "Vinaceous Pink," paler near the apex, sometimes "Vinaceous Tawny," glabrous or somewhat fibrillose, viscid when young or when moist, slender, sometimes tapering downward. Flesh white, turning slightly pinkish.

Spores olivaceous under the microscope, elliptical-fusiform, 8–10–12 × 4–5 μ (10 × 4 μ).

Aug. 6, 1938, C. C. Cheo, Haiyuantze, Kunming (*8042*); July 22, 1942, W. F. Chiu, Shishan, Kunming (*7896*); Aug. 11, 1942, the same place (*7851*); June 1938, W. F. Chiu, Mt. Tsing Cheng, Szechwan (Chiu *304*).

Patouillard in his original diagnosis of this species did not mention the gelatinous membrane and the dotted elements on the cap. Since the species is very common in Yunnan, individuals in different stages of development have been studied. It is found that the plant is covered with a gelatinous veil when young, and with minute granules scattering among the veins on the cap. The reticulation is the result of the shrinking of the gelatinous membrane. Thus the veins on the cap become distinct only when the gelatinous membrane is dried and shrunken. The granules or dotted elements which are very distinct when young can be entirely lost when old. Patouillard's description seems to be based only on a certain stage of development.

BOLETUS PLACIDUS Bon.

Pileus 2–11 cm. in diameter, convex to plane, white, "Buff Yellow" to "Amber Brown," very viscid when moist, shining when dry. Tubes 4–6 mm. long, "Sulphur Yellow," decurrent; pores 1–2.5 mm. across, concolorous with the tubes, usually dotted with cremeous-yellow glandules which become dark brown with age, usually angular, sinuous. Stipe 3–5 cm. long, 10–13 mm. thick, whitish to yellowish, dotted with yellowish glandules which turn brown to black with age, subequal or slightly tapering downward, central or excentric, stuffed. Flesh solid, yellowish to yellow, sometimes pinkish at the base of the stipe.

Spores olivaceous under the microscope, ellipsoid, 6–9(10) × 3–4 $\mu$ (8 × 3 $\mu$).

July 23, 1938, C. C. Cheo, Shishan, Kunming (*7902*); Aug. 1938, Chunghotze, Tali, C. C. Cheo (*7775*); Sept. 1938, C. C. Cheo, Chichushan (*7777* and *7780*); Aug. 24, 1940, F. L. Tai, Tapugi, Kunming (*5546*); Dec. 1941, W. F. Chiu et C. I. Wu, Tiehfungan, Kunming (*7714* and *7739*); June 30, 1941, C. C. Cheo, Miaokaotze, Kunming (*7705*); July 8, 1942, W. F. Chiu, Tapugi, Kunming (*7854, 7870* and *7874*). Under *Pinus yunnanensis*.

Kallenbach thoroughly studied this species and gave excellent paintings (Die Pilze Mitteleuropas B.I.S.: 101, **1923–34**). According to the opinion of Kallenbach, Bonordon's species should not be placed in the genus *Gyrodon*. The color plate given by Bresadola (Icon. Myc. T. 944) is rather poor. The writer has collected this plant under pines for several years, and has been able to study its various stages of development. The Yunnan plant is exactly identical with the European form. However, some young fruiting bodies, as far as observed in the Yunnan collections, do appear to be of the *Gyrodon* type, *i.e.*, with very short and decurrent tubes. The color of the cap varies from white, "Buff Yellow" to "Amber Brown," whereas that of the granules on the stipe is cream-yellow when young, and always becomes black with age. The length of time that a sporophore requires to take for passing from one stage of growth to the other is variable. The granules on the stipes of some specimens may remain cremeous yellow for a very long period, whereas in other cases, such granules soon darken.

BOLETUS GRANULATUS Fr. ex L.

Pileus 2.4–4.5 cm. in diameter, convex to plano-convex, glabrous, viscid, "Vinaceous Pink" to "Chocolate Brown." Tubes 2–3 mm. long, yellow, short, subdecurrent or adnate; pores less than 1 mm. across, concolorous with the tubes, dotted with cremeous granules, simple, angular. Stipe 3–7 cm. long, 6–12 mm. thick, whitish, dotted with dark brown or reddish-brown granules, subequal. Flesh whitish to yellowish in the stipe, unchanging.

Spores olivaceous under the microscope, spindle-shaped or fusiform, 6–9 × 2–3 μ (6 × 2 μ).

Aug. 28, 1938, Chunghotze, Tali, C. C. Cheo (*7802*).

BOLETUS PORPHYROSPORUS Fr.

Pileus 5 cm. in diameter, convex, "Mummy Brown," to "Bister," velvety, rugose; margin slightly incurved or straight. Tubes 3–8 mm. long, "Vandyke Brown," round and free; pores 5–7 mm. across, concolorous with the tubes, angular to roundish. Stipe 11 cm. long, 7 mm. thick, brownish, punctate and streaked with dark ("Vandyke Brown") elements, rather slender. Flesh whitish, becoming pinkish in the stipe, fibrous.

Spores ochraceous or brown under the microscope, elliptical-fusiform, 14–17 × 6–7 μ (16 × 6 μ).

Aug. 28, 1938, S. T. Chao, Shishan, Kunming (*8038*).

This is a slender-stiped form.

**Boletus virens** sp. nov.

Pileo 2.5–8 cm. lato, hemispherico dein convexo, mox prope plano, sicco, glabro, interdum minutissime pruinoso, primitus "Danube Green," "Saccardo Olive," aetate "Primuline Yellow" v. "Mustard Yellow," saepe olivaceo-squamuloso et rimoseo-areolato; tubulis usque ad 20 mm. longis, albidis dein "Light Congo pink," ventricosis v. posteriore angulatis; poris 1–2 mm. latis, aetate majusculis, usque ad 4 mm. latis, "Congo Pink," rotundatis; stipite 2–7 cm. longo, 7–20 mm. crasso, subaequali v. subventricoso, "Pale Chalcedony Yellow," "Pinard Yellow," obscure reticulato v. lateritio-striato v. puniceo-punctato, basi saepe "Salmon Orange" v. "Orange Chrome"; carne flava, immutabili; sporis hyalinis, ellipsoideis, 9–11–14 × 5–6 μ (12 × 6 μ).

Pileus 2.5–8 cm. in diameter, convex to nearly plane, "Danube Green," "Kronberg Green" or "Saccardo's Olive" when young, "Primuline Yellow" to "Mustard Yellow" when old, usually covered with "Olive-Lake" squamules which are not distinct until the pellicle is areolately cracked with age. Tubes up to 2 cm. long,

light "Congo Pink," ventricose to angular posteriorly, free from or depressed around the stipe; pores large, about 1–2 mm. across (4 mm. across when old), concolorous with the tubes, roundish. Stipe 2–7 cm. long, 7–20 mm. thick, "Pale Chalcedony Yellow" or "Pinard Yellow," indistinctly reticulate with "Olive Lake" veins, sometimes tinged reddish in certain portions, sometimes tinged "Empire Yellow" at the basal portion, stuffed. Flesh pale "Pinard Yellow," unchanging.

Spores pale olivaceous under the microscope, elliptical, 11–14 × 5.5–6 μ (12 × 6 μ).

July 23, 1938, Chenkung, C. C. Cheo (*7882*); July 22, 1942, W. F. Chiu, Shishan, Kunming (*7877*); Aug. 7, 1942, W. F. Chiu, Miaokaotze, Kunming (*7859*), **Type.** Usually under *Keteleeria Evelyniana.*

The color of the cap varies much according to the stage of development. Young fruiting bodies with green cap and the old ones usually with yellow to olive cap might be mistaken for two distinct species if the variations were not studied in the field. This species is close to *B. felleus,* but the cap of the latter is never green, so far as the available records show.

**Boletus punctato-fumosus** sp. nov.

Pileo 2–3.5 cm. lato, hemispherico v. pulvinato dein convexo, "Snuff Brown," minute pubescenti; tubulis 7 mm. longis, carneis, ad stipitem depressis; poris angulatis, 1 mm. latis, carneis; stipite 5–6 cm. longo, 7–8 mm. crasso, subaequali, flavo, rufo-brunneo punctato; carne flava; sporis pallidiore olivaceis, ellipsoideis, 9–12 × 5–6 μ (11 × 5 μ).

Pileus 2–3.5 cm. in diameter, hemispherical to pulvinate, becoming flesh-colored, depressed around the stipe; pores less than 1 mm. across, concolorous with the tubes, simple, angular. Stipe 5–6 cm. long, 7–8 mm. thick, "Empire Yellow," punctate with dark-brown elements, subequal, slightly tapering upward. Flesh whitish, yellowish at the base of the stipe, unchanging.

Spores pale olivaceous under the microscope, ellipsoid, 9–11 × 5–6 μ (11 × 5 μ).

Sept. 14, 1938, C. C. Cheo, Chichushan, Binchwan (*7803*), **Type.** Under *Pinus Armandii.*

In the *Tylopilus* group of the boletes, three species of Asia, namely *B. punctato-fumosus, B. javanicus,* and *B. roseolus,* are closely related. The first one can be easily distinguished from the

latter two by the dark "Snuff Brown" cap and the yellow, punctate stipe.

**Boletus roseolus** sp. nov.

Pileo 2–3 cm. lato, convexo v. plano-convexo, pubescenti, "Carnelian Red" v. "Pale Brazil Red," interdum pallescenti; tubulis 3–5 mm. crasso, apice flavido, basi "Empire Yellow" sed sursum e medius "La France Pink," pubescenti; carne flavida; sporis pallidiore olivaceis, 9–14 × 5–6 μ (11 × 5 μ).

Pileus 2–3 cm. in diameter, convex to plano-convex, pubescent, "Carnelian Red" to "Pale Brazil Red," occasionally becoming much paler. Tubes 3–5 mm. long, flesh-colored; pores 1 mm. across, concolorous with the tubes. Stipe 4.5–7 cm. long, 5–10 mm. thick, yellowish at the apex, "Empire Yellow" at the base, sometimes "La France Pink" from the base to the middle, pubescent. Flesh yellowish.

Spores pale olivaceous under the microscope, elliptical, 9–14 × 5–6 μ (11 × 5 μ).

Sept. 7, 1938, H. S. Yao, Chichushan, Binchwan (*7807*), **Type.** Under *Pinus Armandii;* July to Aug. 1938, C. C. Cheo, Kunming (*7891*). Under *Pinus yunnanensis.*

This species is close to the Java plant, *B. javanicus* P. Henn., but is distinct from the latter in the pubescent cap, the yellow and pink stipe and the broader spores.

BOLETUS VELATUS (Rostr.) Sacc.

Pileus 1–3 cm. in diameter, convex, dry, "Auburn" to "Chestnut Brown," rimoso-squamose; margin stellately appendiculate with the extruding edge of the membrane of the cap (or the extruding part wrapping around the stipe). Tubes 5 mm. long, incarnate, free; pores 0.5–1 mm. across, whitish, angular, simple. Stipe 4–8 cm. long, 5–6 mm. thick, "Ochraceous Salmon" above, "Tawny" below, glabrous, equal, stuffed. Flesh white, usually pinkish beneath the pellicle.

Spores elliptical to subfusiform, pale olivaceous under the microscope, 12–16 × 4–6 μ (14–16 × 5 μ).

Aug. 7, 1942, Miaokaotze, Kunming, W. F. Chiu (*8047*); Sept. 4, 1942, the same locality, S. C. Shen (*7850*). Under *Keteleeria Evelyniana.*

The spore measurements of this species are lacking in the original diagnosis. Externally the Yunnan plant appears to be identical with the Siamese plant.

**Boletus albofarinaceus** sp. nov.

Pileo 5 cm. lato, convexo, sicco, albo, albofarinaceo; tubulis 3 mm. longis, "Pinkish Vinaceous" liberis; poris 7–10 mm. latis, "Dark Vinaceous"; stipite 6–7 cm. longo, 7–8 mm. crasso, aequali v. deorsum ventriculoso, albo, brunneo-striato, basi albido; carne alba, basi flavida, immutabili; sporis hyalinis, ellipsoideis, 11–14 × 5–7 μ (12 × 6 μ).

Pileus 5 cm. in diameter, convex, dry, white, white-pulverulent. Tubes 3 mm. long, "Pinkish Vinaceous," free from the stipe, ventriculose; pores 0.7–1 mm. across, "Dark Vinaceous," simple, angular. Stipe 6–7 cm. long, 7–8 mm. thick, white, yellowish at the base, pulverulent, striate with brown fibrils, slightly dilated toward the base. Flesh whitish, fibrous, yellowish at the basal part of the stipe, unchanging.

Spores hyaline under the microscope, ellipsoid, 11–14 × 5–7 μ (12 × 6 μ).

Aug. 28, 1938, Kunming, F. L. Tai (*8037*), **Type.**

This plant is obviously of the *Tylopilus* type. However among boletes of this group, species with white and white-pulverulent caps have not been recorded.

BOLETUS UMBRINUS Pers.

Pileus 2–8 cm. in diameter, pulvinate, becoming plano-convex, "Russet" to "Pecan Brown" or "Vandyke Brown," subglabrous, rimoso-areolate with age; margin wavy. Tubes 5–8 mm. long, whitish, adnate to subdecurrent; pores 5–8 cm. across, whitish becoming brown, angular, simple. Stipe 3.5–9 cm. long, 1–4 cm. thick, "Pecan Brown" with dark-brown-reticulated veins at the apex, ventricose when old, thick. Flesh solid, whitish, slightly pinkish when exposed to the air.

Spores olivaceous under the microscope, elliptical to subfusiform, 8–12 × 3–5 μ (11 × 4 μ).

Aug. 11, 1941, Tapugi, Kunming, C. C. Cheo (*7694*); Aug. 20, 1942, the same locality, W. F. Chiu (*7857*); July 22, 1942, Shishan, Kunming, W. F. Chiu (*7869*).

According to the original diagnosis, *B. umbrinellus* Pat. et Baker does not seem to differ from *B. umbrinus* except that the spores are narrower. The width of the spores of the Yunnan plant lies between these two species, *i.e.*, 8–12 × 3–5 μ. The writer is of the opinion that *B. umbrinellus* might be synonymous with *B. umbrinus*.

BOLETUS CROCIPODIUS Letel.

Pileus 7–14 cm. in diameter, hemispherical, becoming convex then plane and reflexed, somewhat viscid when moist, "Mars Yellow," tomentose, rugose when young, rimoso-areolate with age; margin extended beyond the tubes forming a narrow collar closely applied to the stipe when young. Tubes 8–23 mm. long, "Empire Yellow," becoming "Citron Green" with age, usually free; pores minute, less than 1 mm. across, concolorous with the tubes. Stipe 5–12 cm. long, 2.5–4.5 cm. thick, "Antimony Yellow," punctate with concolorous elements, equal or slightly tapering upward, rarely tapering downward, stuffed. Flesh solid, white, changing to lilac in the stipe, yellowish above the tubes when exposed to the air.

Spores olivaceous under the microscope, elliptical to ellipsoid-oblong, 9–12–(14) × 3–5 μ (11 × 4 μ).

July 1941, Tapugi, F. L. Tai et C. C. Cheo (*7883, 7749, 7755, 7741* and *7738*); July 11, 1942, Tapugi, Kunming, Mrs. Chiu (*7829*).

The present fungus is very close to *B. rimosus* (*B. tesselatus* etc.) except that it has smaller spores. It is also distinct from *B. fuscescens* Clel., which may be synonymous with *B. crocipodius*, in the different color and the non-bulbous stipe.

Gilbert maintains that *Krombholzia crocipodia* (Letel.) Gilb. should be the right name, and points out at the same time that *B. rimôsus* Vent. is distinct from the former (Les Bolets: 179, **1931**). Singer, however, considers *K. luteopora* (Bouchinot) as the right name with *K. crocipodia* as a synonym. His reason for the change is that before the publication of *B. luteoporus*, there was no valuable diagnosis of *B. crocipodius* (Rev. Myc. **3**: 188, 1938). In Singer's recent publication, *K. luteopora* has been made a synonym of *Leccinum nigrescens* (The Boletineae of Florida III: 117, **1947**). In spite of all such changes, the writer is using the present name, because it has been generally accepted.

**Boletus violaceo-fuscus** sp. nov.

Pileo 4–7 cm. lato, semigloboso, dein convexo, prope plano, "Blue Violet" v. "Maroon Purple," mox pallescenti, raro rugoso, minutissime velutino; tubulis 3–6 mm. longis, albis dein flavidis, sinuatis v. liberis; poris minutis, 1–2 in uno mm. latis, albis, aetate flavidis; stipite 5–7 cm. longo, 1–2 cm. crasso, subaequali, basi ventricoso, pilei concolore, reticulato; carne alba, immutabili; sporis olivaceis, fusoideo-ellipticis, 12–14 × 5–6 μ (12 × 6 μ).

Pileus 4–7 cm. in diameter, hemispherical, becoming plano-convex, dark purple to dark "Blue Violet," sometimes fading to dark "Maroon Purple," usually smooth, rarely rugose, minutely velvety. Tubes 3–6 mm. long, at first white, becoming yellowish, sinuate or free, depressed around the stipe; pores minute, 0.5–1 mm. across, white becoming yellowish with age or when bruised. Stipe 5–7 cm. long, 1–2 cm. thick, subequal or ventricose at the basal portion, concolorous with the cap but often paler, distinctly reticulate with white veins. Flesh white, solid, becoming spongy when old or when tunneled by worms.

Spores olivaceous under the microscope, fusiform-elliptical, 12–14 × 5–6 μ (12 × 6 μ).

Sept. 2, 1938, S. T. Chao, Kunming market (*7007*, **Type,** and *7008*); July 8, 1938, Chengtu market, W. F. Chiu (Chiu *74*); Aug. 1942, the writer encountered this plant in *Quercus* woods (*Q. variabilis*). The size of cap reaches 25 cm. in diameter, and the stipe is as thick as 4 cm. Unfortunately this specimen had collapsed before it could be delivered to the laboratory for preservation.

This plant is distinct among the white-tubed boletes for its dark violet color and the beautiful white reticulation on the stipe. Specimens were first collected from the Chengtu market where it is known as edible. Studies in detail and descriptions were not made until it was encountered again in the Kunming market. Its association with *Quercus variabilis* was recognized in a field near Tapugi, Kunming.

BOLETUS AEREUS Fr. ex Bull.

Pileus 3–11 cm. in diameter, hemispherical, becoming convex and plano-convex, dry, minutely velvety or becoming glabrous, dark "Chestnut Brown," "Natal Brown" to "Drab," nearly "Pale Pinkish Buff" in some cases; margin sometimes wavy. Tubes 4–8 mm. long, white becoming "Vinaceous Buff," free or uncinate; pores 0.5–1 mm. across, grayish-white, simple, round, uneven. Stipe 3–9 cm. long, 1.5–5 cm. thick, "Medal Bronze," "Clay Color" or "Mikado Brown," paler in the upper part, distinctly reticulate with dark-brown veins, ventricose when young, subequal when old, central or excentric. Flesh firm, white, sometimes turning pinkish when exposed to the air or becoming yellow where bruised or tunneled by worms, rarely changing to bluish at the base of the stipe.

Spores olive under the microscope, oblong-fusiform or oblong-elliptical, 9–12 × 4–5 μ (11 × 4 μ).

July 13, 1938, Kunming market, C. C. Cheo et S. T. Chao (*5481, 5568* and *7898*); July to Aug. 1941, Tapugi, Kunming, F. L. Tai et C. C. Cheo (*7747, 7754, 7742, 7463, 7894* and *7723*). Usually under *Quercus variabilis*.

The variation of the cap color has been carefully observed in the Yunnan plant. The color ranges from dark "Chestnut Brown," "Natal Brown," "Drab" to nearly "Pale Pinkish Buff." The white tubes do not change to yellowish or yellowish-green but to "Vinaceous Buff." The stipe color varies from "Medal Bronze," "Clay Color" to "Mikado Brown."

BOLETUS RAVENELII Berk. et Curtis

Pileus 3–6 cm. in diameter, hemispherical to convex, then slightly depressed, yellow, sometimes tinged pinkish or reddish, generally yellow-pulverulent; margin appendiculate with yellow membrane. Tubes 6–9 mm. long, yellow, becoming ochraceous; pores more or less 1 mm. across, "Aniline Yellow" becoming ochraceous, angular. Stipe 3–9 cm. long, 0.5–1 cm. thick, concolorous with the cap but paler above the ring, farinaceous, equal, stuffed, sometimes tinged reddish. Flesh whitish, turning bluish when exposed to the air.

Spores pale "Lumiere Green" under the microscope, ellipsoid, 8–11(12) × 4.5–5.5 μ (9 × 4.5 μ).

Sept. 4, 1938, Kunming market, F. L. Tai (*7701*); July 28, 1938, Mt. Omei, Szechwan (*7688*). Under coniferous tree.

**Boletus pseudostrobilomyces** sp. nov.

Pileo 6.5 cm. lato, semigloboso, sicco, "Vinaceous Cinnamon" v. "Cinnamon," strobilaceo-squarroso, "Verona Brown"-squamoso; tubulis 15 mm. longis, "Lemon Yellow," adnatis; poris mediis, concolore, rotundatis; stipite 14 cm. longo, 8 mm. crasso, "Cinnamon," "Russet," fibrilloso, aequali, farcto; carne alba, caerulescente; velo griseo, ad marginem appendiculato; sporis ochraceis, ellipsoideis, 15–19 × 7.5–8 μ.

Pileus 6.5 cm. in diameter, hemispherical, dry, "Vinaceous Cinnamon," covered with thick, "Verona Brown," strobilaceous scales. Tubes 15 mm. long, "Lemon Yellow," adnate; pores moderately large, concolorous with the tubes, roundish. Stipe 14 cm. long,

8 mm. thick, "Cinnamon," minutely striate with "Russet" fibrils, equal, pithed. Flesh white changing immediately to blue when exposed to the air. Veil grayish, membranous, persistent and appendiculate to the margin of the cap.

Spores smooth, brown or dark ochraceous under the microscope, elliptical, 15–19 × 7.5–8 μ.

Aug. 2, 1938, W. F. Chiu, Mt. Omei, Szechwan (Chiu *121*), **Type.** Under *Machilus bournei.*

The pyramidal scales on the cap of this fungus are somewhat like those of *Strobilomyces floccopus,* but according to the spore-form and other macroscopical characters, it belongs evidently to the *Xerocomus* group. The Australian species *S. velutipes* has been described as having smooth or partially smooth spores. However, the present plant differs from the Australian species in the entirely different colors, the non-blackening of the flesh, and the smooth, elliptical and longer spores.

**Boletus subpaludosus** sp. nov.

Pileo 3.5–4 cm. lato, convexo, "Pale Vinaceous Tawny," glabro; tubulis 4–5 mm. longis, flavidis v. flavis, caerulescentibus, adnatis v. breviter decurrentibus; poris flavis, 0.8–1 mm. latis, angulatis; stipite 4–8 cm. longo, 4–6 mm. crasso, subaequali, "Light Vinaceous Cinnamon" v. "Cinnamon," glabro, saepe striato v. leniter flexuoso; carne flava, caerulescente; sporis fusco-olivaceis, ellipsoideis, 8–12 × 4–5 μ (11 × 4.5 μ).

Pileus 3.5–4 cm. in diameter, convex, "Pale Vinaceous Tawny," glabrous. Tubes 4–5 mm. long, yellow, changing to blue when bruised, sinuate, decurrent with a short tooth; pores concolorous with the tubes, 0.8–1 mm. across, angular to somewhat labyrinthiform. Stipe 4–8 cm. long, 4–6 mm. thick, subequal or slightly tapering near the base, "Light Vinaceous Cinnamon" to "Cinnamon," glabrous, usually striate, slightly curved. Flesh yellow, changing to blue when exposed to the air.

Spores dark olive under the microscope, elliptical or ellipsoidal, 8–12 × 4–5 μ (11 × 4.5 μ).

Aug. 1940, Tapugi, Kunming, F. L. Tai (*8033*), **Type.**

This plant appears macroscopically to be close to *B. paludosus,* but differs from the latter in that its flesh and tubes change to blue when bruised. It also differs from *B. yunnanensis* in the changing flesh and the glabrous cap.

BOLETUS COMMUNIS Bull. var. MUTATIS Schultz

Pileus 3–3.5 cm. in diameter, convex to nearly plane, "Buffy Brown," "Yellow Olive," with minute tomentum which is tinged "Saccardo Olive," usually finely areolate, reddish at the wounded spot on the cap. Tubes "Amber Yellow," ventricose, up to 6 mm. long, free or uncinate, deeply depressed around the stipe with age, slowly changing to green when bruised; pores around 1 mm. across, concolorous with the tubes. Stipe 3–3.5 cm. long, 4–5 mm. thick, slightly tapering downward, yellow at the apex, "Argus Brown" below, striate with reddish fibrils. Flesh white, reddish beneath the cuticle, yellowish in the middle, slowly changing to blue in the cap and to reddish in the stipe when exposed to the air.

Spores olivaceous under the microscope, elliptical, 9–11 × 4–5 $\mu$ (11 × 4.5 $\mu$).

June 20, 1943, S. Y. Yin, Chiunchutze, Kunming (*8200*). In deciduous woods on damp slope.

The typical form of this species has not been collected in this province. *B. chrysenteron* Bull., which is a synonym of *B. communis,* has been recorded by Teng (S. C.) from Kiangsu.

BOLETUS TOMENTIPES Earle

Pileus 3.5–11 cm. in diameter, convex to plane, "Brussels Brown," minutely tomentose, becoming subglabrous, sometimes rimose-areolate. Tubes about 1 cm. long, "Antimony Yellow," free to uncinate, depressed around the stipe; pores minute, less than 1 mm. across, concolorous with the tubes, somewhat angular. Stipe 5–8 cm. long, 15–20 mm. thick, "Light Coral Red" at the apex, "Brussels Brown" downward, minutely tomentose, subequal, stuffed. Flesh whitish or brownish, changing to blue when bruised.

Spores dark olive under the microscope, elliptical, 9–12 × 5–6 $\mu$ (11 × 5.5 $\mu$).

Sept. 13, 1938, Chichushan, Binchwan, C. C. Cheo (*7804*).

This form accords closely with the original description of *B. tomentipes* except that the tubes do not turn red when bruised.

**Boletus nigropunctatus** sp. nov.

Pileo 6–7 cm. lato, hemisphaerico, "Saccardo's Umber," fusco-brunneo- v. "Sepia"-punctato, tubulis usque ad 12 mm. longis, flavis dein ochraceis, ad stipitem depressis; poris majusculis, hexagonalibus, flavis dein ochraceis;

stipite 6–7 cm. longo, 5–8 mm. crasso, pileo subconcolore; saepe pallidiore, glabro, aequali; carne flavida, caerulescente; sporis ochraceis, ellipsoideis, 6–8 × 3–4 μ.

Pileus 6–7 cm. in diameter, hemispherical, "Saccardo's umber," punctate with dark brown or "Sepia" elements, dry. Tubes 12 mm. long, yellowish, becoming ochraceous with age, convex, depressed around the stipe; pores large, hexagonal, concolorous with the tubes. Stipe 6–7 cm. long, 5–8 mm. thick, subconcolorous with the cap, usually paler, equal, stuffed. Flesh yellowish, changing to blue when exposed to the air.

Spores ochraceous under the microscope, ellipsoidal, 6–8 × 3–4 μ.

Aug. 4, 1938, Mt. Omei, W. F. Chiu (Chiu *262*), **Type.** In coniferous woods.

**Boletus instabilis** sp. nov.

Pileo 6–7 cm. lato, pulvinato, obliquo, "Ochraceous Tawny," subglabro, tesselato-areolato; tubulis usque ad 10 mm. longis, flavis, rotundatis; stipite 6–7 cm. longo, 1.5–2.5 cm. crasso, "Pinkish Cinnamon," glabro, deorsum attenuato, crasso, excentrico; carne alba, fracta pilei punicea, stipiti brunneo-striata; sporis olivaceis, ellipsoideis v. subfusiformibus, 9–14 × 4.5 μ (11 × 4.5 μ).

Pileus 6–7 cm. in diameter, pulvinate, sloping, "Ochraceous Tawny," subglabrous, tesselately areolate; margin notched at a point. Tubes up to 10 mm. long, yellow, changing to blue when bruised, somewhat short-decurrent; pores 0.5–1 mm. across, concolorous with the tubes, roundish. Stipe 6–7 cm. long, 15–25 mm. thick, extremely excentric to nearly lateral. Flesh white, pinkish in the cap and streaked with brown fibers in the stipe when cut.

Spores olive under the microscope, elliptical to subfusiform, 9–14 × 4–5 μ (11 × 4.5 μ).

July 23, 1938, C. C. Cheo, Chenkung (*7895*), **Type.**

The present fungus differs from *B. paludosus* Mass. in the nearly lateral stipe and the always tesselately areolate cap.

**Boletus Cheoi** sp. nov.

Pileo 1.5–5 cm. lato, pulvinato dein convexo, saepe subumbonato, sicco, primitus "Liver Brown," aetate "Cinnamon Rufous," fusco-brunneo-fibrilloso e squamuloso; tubulis 4–12 mm. longis, "Amber Yellow" v. "Citron Yellow," flavidis, caerulescentibus, adnatis; poris 1–2 mm. latis, flavis; stipite 3–6 cm. longo, 1–7 mm. crasso, cylindrico, saepe sursum attenuato, "Pinkish Buff" v. "Tawny Olive," apice pallidiore caerulescente; sporis olivaceis, ellipsoideis, 8–11 × 4–5 μ (9 × 5 μ).

Pileus 1.5–5 cm. in diameter, pulvinate then convex, subumbonate, dry "Liver Brown" when young, "Cinnamon Rufous" with age, covered with dark brown fibrillose scales. Tubes 4–12 mm. long, "Amber Yellow" or "Citron Yellow," turning blue when bruised, adnate; pores 1–2 mm. across, concolorous with the tubes. Stipe 3–6 cm. long, 1–7 mm. thick, slender, usually tapering upward, "Pinkish Buff" or "Tawny Olive" at the top, glabrous, stuffed. Flesh whitish turning brownish or reddish when exposed to the air.

Spores olivaceous under the microscope, elliptical, 8–11 × 4–5 $\mu$ (9 × 5 $\mu$).

Sept. 11, 1938, Chichushan, Binchwan, C. C. Cheo (*7692*). **Type;** Aug. 24, 1938, Tali, C. C. Cheo (*7700*); July 19, 1938, Shishan, Kunming, T. K. Yien (*7698* and *7699*); Aug. 1938, the same locality, F. L. Tai et C. C. Cheo (*7697* and *7715*); July 12, 1943, Shishan, Kunming, W. F. Chiu (*8025*). Usually in woods of *Keteleeria Evelyniana*.

In Yunnan there are two small boletes of the *Xerocomus* type: *B. Cheoi* and *B. punctilifer*. They are both close to *B. decorus*, but can be readily distinguished from the latter by the yellow and pinkish flesh, and the shorter spores which never reach 13 $\mu$ long. The present species can also be separated from *B. punctilifer* by the umbonate cap, the broad adnate tubes and the glabrous stipe.

**Boletus punctilifer** sp. nov.

Pileo 3–8 cm. lato, convexo dein plano, sicco, punctato-tomentoso, primitus "Argus Brown," "Liver Brown" dein "Hay's Russet" v. "Cinnamon Rufous"; tubulis 10 mm. longis, "Amber Yellow" v. "Citron Yellow," sinuatis; poris circa 1 mm. latis, angulatis, leviter puniceis dein flavis, aetate cyanescentibus; stipite 4–7 cm. longo, 7–10 mm. crasso, subaequali v. subventricoso, supra "Pinkish Cinnamon," infra flavido, minute punctato e fibrilloso, glabrescenti, farcto; carne pilei flavida, stipiti punicea; sporis breviter ellipsoideis, olivaceis, 7–10 × 4.5–5.5 $\mu$ (9 × 4.5 $\mu$).

Pileus 3–8 cm. in diameter, convex becoming plane, punctate with small tufts of tomentum, "Argus Brown" or "Liver Brown," becoming "Hay's Russet" to "Cinnamon Rufous," dry. Tubes 10 mm. long, "Amber Yellow" to "Citron Yellow," sinuate; pores around 1 mm. across, angular, slightly pinkish then yellowish, turning blue with age. Stipe 4–7 cm. long, 7–10 mm. thick, subequal to ventricose, "Pinkish Cinnamon" in the upper part, yellow below, finely punctate and fibrillose, becoming glabrous when old, solid. Flesh yellowish in the cap, pinkish in the stipe.

Spores short ellipsoid, olivaceous under the microscope, 7–10 × 4.5–5.5 μ (9 × 4.5 μ).

June 21, 1941, Miaokaotze, Kunming, C. C. Cheo (*7735*); Aug. 1938, Shishan, Kunming, W. F. Chiu (*7748*); July 8, 1942, Tapugi, Kunming, W. F. Chiu (*7860,* **Type,** and *7873*).

This plant is closely related to *B. Cheoi* except that the convex pileus is never umbonate, and the punctate stipe never smooth when young. Another character which serves to separate these two species is the attachment of the tubes. In the present fungus, the tubes are always free from the stipe, while in *B. Cheoi* they are broadly adnate.

**Boletus puniceus** sp. nov.

Pileo 5 cm. lato, plano, "Old Rose," minute tomentoso; tubulis usque ad 17 mm. longis, "Pinard Yellow," immutabilibus, sinuatis; poris majusculis, 2–2.5 mm. latis, flavis, rotundatis e angulatis; stipite 11 cm. longo, 8–12 mm. crasso, leviter sursum attenuato, pileo concolore, dense floccoso e pubescenti; carne alba, immutabili; sporis olivaceis, ellipticis, 12–19 × 7–8 μ (16 × 8 μ).

Pileus 5 cm. in diameter, plane, "Old Rose," minutely tomentose. Tubes up to 17 mm. long, "Pinard Yellow," unchanging, sinuate; pores large, 2–2.5 mm. across, concolorous with the tubes, roundish to angular. Stipe 11 cm. long, 8–12 mm. thick, concolorous with the cap, densely floccose and pubescent, slightly tapering upward. Flesh white, yellowish at the base of the tube layer, unchanging.

Spores olivaceous under the microscope, elliptical, large, 12–19 × 7–8 μ (16 × 8 μ).

Sept. 30, 1942, Miaokaotze, Kunming, H. R. Wang et K. S. Wu (*7825*), **Type.**

The distinguishing characters of the species are the large spores which are rarely found in species of the *Xerocomus* group, and the large angular pores which are also rare in the related species.

**Boletus yunnanensis** sp. nov.

Pileo 2.2–3.8 cm. lato, convexo, "Argus Brown," velutino; tubulis "Lemon Yellow" dein "Aniline Yellow," 4–5 mm. longis, breviter decurrentibus; poris 0.7–1 mm. latis, aetate ochraceis, angulatis; stipite 3–5 cm. longo, 3–10 mm. crasso, aequali v. leniter sursum attenuato, basi leviter bulboso, "Avellaneous," glabro; carne pilei flava, stipiti alba; sporis olivaceis, ellipsoideis v. subellipticis, 7.5–11 × 3–4.5 μ (9 × 4.5 μ).

Pileus 2.2–3.8 cm. in diameter, convex, "Argus Brown, distinctly velvety. Tubes "Lemon Yellow," becoming "Aniline Yellow," 4–5 cm. long, decurrent with a short tooth; pores 0.7–1 mm. across, concolorous with the tubes, turning ochraceous with age, angular, somewhat irregular. Stipe 3–5 cm. long, 3–10 mm. thick, equal or slightly tapering upward, slightly bulbous at the base. Flesh yellow in the cap, white in the stipe.

Spores slightly olivaceous under the microscope, elliptical to subelliptical, 7.5–11 × 3–4.5 μ (9 × 4.5 μ).

July, 1938, Shishan, Kunming, F. L. Tai (*7900*), **Type.**

This species is usually confused with *B. subpaludosus* especially when dry, but the velvety cap of the former can still be recognized even when dried in contrast with the glabrous cap of *B. subpaludosus*. The unchanging flesh and the short spores of the present species also serve to separate it from *B. paludosus* Mass.

BOLETUS SYLVESTRIS ? Petch

Pileus 6 cm. in diameter, rarely plane, "Vinaceous Rufous," "Bay" at the center, minutely tomentose, becoming glabrous. Tubes 6 mm. long, yellow, adnate-uncinate; pores about 1 mm. across, concolorous with the tubes, angular, simple. Stipe 3.5 cm. long, 1 cm. thick, "Tawny," tomentose, paler toward the base, slightly ventricose. Flesh white, turning slightly pinkish.

Spores olivaceous under the microscope, elliptical, 7.5–11 × 4.5–6.5 μ (9 × 5 μ).

July 31, 1938, Chiunchutze, Kunming, C. C. Cheo (*8036*).

This plant is externally close to *B. sylvestris* Petch, but as the shape and size of the spores of the latter were not given by Petch in his original diagnosis, the writer cannot be certain that the Ceylon plant is exactly identical with ours. The spores of the present plant are olivaceous under the microscope and elliptical in shape—features usually considered as characteristic for the *Xerocomus* group. The name is applied to this collection only tentatively.

BOLETUS UNICOLOR ? Frost

Pileus 6–8 cm. in diameter, convex to plane, slightly viscid when moist, subglabrous; "Mustard Yellow" or "Primuline Yellow," darker with age. Tubes 3–6 mm. long, rather short when young, concolorous with the cap, becoming "Ochraceous Buff," free; pores

0.5–1 mm. across, concolorous with the tubes, angular. Stipe 7–9 cm. long, 13–20 mm. thick, concolorous with the cap, glabrous, subequal. Flesh pale "Amber Yellow," unchanging.

Spores olivaceous under the microscope, ellipsoid, 9–12 × 5–6 μ (11 × 5 μ).

Oct. 13, 1938, Shishan, Kunming, C. C. Cheo (*7716*).

**Boletus rugosellus** sp. nov.

Pileo 4.5–11 cm. lato, convexo dein plano-convexo, sicco, glabro et nitido, primitus rugoso, mox laevi, "Tawny," tubulis 5–7 mm. longis, "Green Yellow," ochrascentibus, adnatis v. liberis; poris 2 in uno mm., "Green Yellow," angularibus; stipite 8–15 cm. longo, 7–14 mm. crasso, subaequali v. sursum attenuato, apice flavido, infra "Shell Pink" v. "Japanese Rose," obscuriore brunneo-striato, interdum albo-pubescenti; carne flavida, immutabili; sporis olivaceis, ellipticis, 9–12(17) × 4.5–5.5 μ (11 × 4.5 μ).

Pileus 4.5–11 cm. in diameter, convex then nearly plane, dry, glabrous and shining, at first rugose, becoming smooth, "Tawny." Tubes 5–7 mm. long, "Green Yellow," free; pores 0.5 mm. across, concolorous with the tubes, simple, angular. Stipe 8–15 cm. long, 7–14 mm. thick, yellowish at the apex, "Shell Pink" downward, streaked with brownish fibrils, sometimes tinged "Japanese Rose" and covered with white down, subequal, slightly tapering upward. Flesh whitish to yellowish, unchanging.

Spores olive under the microscope, elliptical, 9–12(17) × 4.5–5.5 μ (11 × 4.5 μ).

July 22, 1942, Shishan, Kunming, W. F. Chiu (*7872*), **Type.** Under *Keteleeria Evelyniana;* Sept. 1942, the same locality (*8245*).

This fungus is close to *B. recedens* but of different color. The longer spores of the present plant are also characteristic.

BOLETUS RUBROPUNCTUS Peck

Pileus 3.5 cm. in diameter, convex, "Sanford Brown" to "Burnt Sienna," subglabrous, dry. Tubes up to 7 mm. long, "Pinard Yellow," unchanging, free; pores small, about 0.5 mm. across, roundish, concolorous with the tubes. Stipe 8 cm. long, 3.5 cm. thick, "Baryta Yellow," punctate with reddish brown elements at the apex, streaked with reddish-brown fibrils downward, subventricose, tapering at the base. Flesh yellowish, unchanging.

Spores pale greenish olive under the microscope, long-elliptical, 11–14 × 4–4.5 μ (14 × 4.5 μ).

July 12, 1943, Shishan, Kunming, W. F. Chiu (*8204*).

**Boletus Taianus** sp. nov.

Pileo 5–7 cm. lato, semigloboso dein plano-convexo, glabro, "Pale Olive Buff"; tubulis 5–8 mm. longis, flavis, fractis caerulescentibus, adnatis; poris 1 mm. latis, "Ox-blood Red" v. "Carmine," angularibus; stipite 6–8 cm. longo, 15–20 mm. crasso, leniter sursum attenuato, supra "Begonia Rose," infra brunneo, reticulato; carne firma, albida, fracta pilei caerulescente, stipite punicea; sporis pallidiore olivaceis v. hyalinis, ellipticis v. subfusiformibus, 8–9 × 3–4 μ (9 × 3 μ).

Pileus 5–7 cm. in diameter, hemispherical becoming plano-convex, glabrous, "Pale Olive Buff"; margin usually wavy. Tubes 5–8 mm. long, yellow, turning blue when bruised, adnate; pores "Ox-blood Red" or dark "Carmine," about 1 mm. across, rather angular. Stipe 6–8 cm. long, 15–20 mm. thick, brown at the basal portion, "Begonia Rose" upward, reticulate at the upper half of the stipe, slightly tapering upward. Flesh firm, white, turning bluish in the cap and pinkish in the stipe, rather fibrous.

Spores pale olivaceous or rather hyaline under the microscope, elliptical to subfusiform, 8–9 × 3–4 μ (9 × 3 μ).

July, 1938, C. C. Cheo from Kunming market (*8308*), **Type.**

*B. Satanas* is a species closely related to the present fungus, but the non-bulbous stipe and the grayish cap which is never tinged greenish, are two external characters for separating *B. Taianus* from *B. Satanas*. The small spores of *B. Taianus* are also characteristic.

**Boletus sinicus** sp. nov.

Pileo 9–11 cm. lato, pulvinato, fibrilloso-squamoso, "Garnet Brown"; tubulis 4 mm. longis, concavis, uncinatis, "Maize Yellow," fractis caerulescentibus; poris minutis, 1–2 in uno mm., "Ox-blood Red." Stipite 8–9 cm. longo, 13–36 mm. crasso, aequali, basi leniter bulboso, pileo concolore, apice flavo, reticulato; carne alba, stipiti flavida, fracta caerulescente; sporis pallidiore olivaceis, breviter ellipsoideis, 7.5–11 × 4.5–5.5 μ (9 × 4.5 μ).

Pileus 9–11 cm. in diameter, pulvinate, fibrillose scaly, "Garnet Brown"; margin paler in color and wavy. Tubes 4 mm. long, concave, uncinate, "Maize Yellow," turning blue when cut; pores small, less than 0.5 mm. across, "Ox-blood red." Stipe 8–9 cm. long, 13–36 mm. thick, equal, slightly dilated at the base, concolorous with the cap or paler, yellow at the apex, reticulate with prominent red veins. Flesh white, yellowish, turning blue when cut.

Spores light olivaceous under the microscope, short ellipsoid, 7.5–11 × 4.5–5.5 μ (9.5 × 4.5 μ).

July 1938, F. L. Tai from Kunming market (*8035*), **Type.**

This species is very close to *B. Frostii* and *B. magnificus*. The present fungus differs however from the American species in the scaly pileus, and from *B. magnificus* in the completely reticulate stipe and shorter spores.

BOLETUS QUELETII Schulz.

Pileus 5–8 cm. in diameter, hemispherical to convex, dry, glabrous, shining, "Cinnamon Brown" to "Brick Red" or "Auburn." Tubes 5–6 mm. long, yellow, sinuate-free; pores pale "Brazil Red" becoming "Carrot Red" tinged yellow, minute, less than 0.5 mm. across, changing to green when bruised. Stipe 6–9 cm. long, 3–5 cm. thick, subequal or bulbous, yellow at the apex, pinkish downward, becoming brown with age, slightly reticulate with red veins on the upper portion. Flesh yellow when young, changing to green in the upper portion and lilac in the lower. Flesh of maturing sporophores white in the cap and yellow in the stipe, changing slowly to green where bruised.

Spores olivaceous under the microscope, elliptical, 10–16 × 5–6 $\mu$ (11 × 5 $\mu$).

July 8, 1942, Tapugi, Kunming, W. F. Chiu (*7875* and *7847*); Aug. 1938, W. F. Chiu from Chengtu market (Chiu *54*).

**Boletus magnificus** sp. nov.

Pileo 5–11 cm. lato, pulvinato dein convexo, "Nopal Red," "Ox-blood Red" v. "Garnet Brown," sicco, interdum "Coral Red," tomentoso, mox glabrescenti; tubulis 7–12 mm. longis, "Lemon Chrome," aetate ochrascentibus, fractis caerulescentibus, liberis; poris minutis, 1–2 in uno mm., "Brazil Red," aetate "Bittersweet Orange," rotundatis v. plus minus angularibus; stipite 5–15 cm. longo, 2–6 cm. crasso, subaequali, basi leniter bulboso, supra "Capucine Yellow," infra pileo concolore, rubro-punctato v. striato, apice raro reticulato, centrico v. excentrico; carne "Pinard Yellow," caerulescente; sporis pallidiore olivaceis, ellipticis, 9–13 × 4–6 $\mu$ (11 × 4–5 $\mu$).

Pileus 5–11 cm. in diameter, pulvinate or convex, "Nopal Red," "Ox-blood Red" or "Garnet Brown," dry, sometimes tinged "Coral Red" or becoming paler, tomentose, sometimes becoming glabrous; margin incurved when young then straight, sometimes wavy. Tubes 7–12 mm. long, "Lemon Yellow" to "Lemon Chrome," becoming ochraceous with age, changing to blue when cut, free; pores minute, 0.5–1 mm. across, "Brazil Red," becoming "Bittersweet Orange" with age, roundish or more or less angular. Stipe 5–15 cm. long, 2–6 cm. thick, "Capucine Yellow" in the upper

part, concolorous below, punctate with red-dotted elements or streaked with red fibrils, or rarely reticulate with red veins at the apex, subequal or slightly bulbous at the base, central or excentric. Flesh "Pinard Yellow," turning immediately blue from outside inward, especially in the cap, when exposed to the air.

Spores pale olivaceous under the microscope, elliptical to fusiform-elliptical, 9–13 × 4–6 μ (11 × 4.5 μ).

July 6, 1938, C. C. Cheo from Kunming market (*7886*); Aug. 1941, Tapugi, Kunming, F. L. Tai (*7893, 7693, 7712* and *7719*, **Type**); Aug. 3, 1938, S. T. Chao from Kunming market (*7727*); Aug. 1942, W. F. Chiu (*7744*); July 9, 1943 (*8207*); June 16, 1942, Miaokaotze, Kunming, W. F. Chiu (*7866*). Under *Pinus Armandii*.

This red bolete is very close to *B. Queletii* and is also easily confused with *B. Frostii* and *B. sinicus*, but it is never entirely reticulate on the stipe as in the case of *B. sinicus*. The color of the cap and of the stipe changes to dirty brown or even blackish on touch, and the color of the pores is usually very dark "Brazil Red." The intensity of the red color fades as the age advances. The orange color of the pores in the later stages of development is likely to lead one to consider such stages as a distinct variety.

**Boletus subsplendidus** sp. nov.

Pileo 2.5–6 cm. lato, convexo dein plano, sicco, minute tomentoso, "Burnt Sienna" v. "Bay," saepe ad marginem flavo-maculato, tacto caerulescenti; tubulis primitus brevissimis, aetate usque ad 7 mm. longis, "Empire Yellow," adnate subdecurrentibus; poris minutis, concolore, fractis caerulescentibus; stipite 6–9 cm. longo, 15–20 mm. crasso, subaequali v. subventricoso, striato e pubescenti, interdum reticulato, tacto brunnescenti; carne flava, caerulescente; sporis olivaceis, ellipsoideis, 9–12 × 4–5 μ (9 × 4.5 μ).

Pileus 2.5–6 cm. in diameter, convex to nearly plane, dry, minutely tomentose, "Burnt Sienna" to "Bay," usually maculate or bordered with "Empire Yellow" at the marginal region, turning bluish when bruised. Tubes very short when young, up to 7 mm. long at maturity, "Empire Yellow," adnate-subdecurrent; pores minute, concolorous with the tubes, turning blue when exposed to the air. Flesh yellow, changing to bluish in the cap and greenish in the stipe. Stipe 6–9 cm. long, 15–20 mm. thick, "Empire Yellow," usually lateritious in the lower part, subventricose or subequal, streaked and pubescent, sometimes obscurely reticulate, turning brown when bruised.

Spores olive under the microscope, ellipsoid, 9–12 × 4–5 μ (9 × 4.5 μ).

July 12, 1942, W. F. Chiu, Miaokaotze, Kunming (*7876*), **Type.** In mixed woods of *Pinus yunnanensis, Keteleeria Evelyniana* and *Lithocarpus* sp.

This plant is close to *B. splendidus* Mart. except that the flesh of the former never changes to red or purple when exposed to the air, and that the yellow stipe is streaked, pubescent and occasionally obscurely reticulate.

BOLETUS ALBIDUS Roq.

Pileus 6–16 cm. in diameter, hemispherical becoming convex, usually slightly depressed at the disk, dry, glabrous, pale "Chestnut Brown" or "Warm Sepia," sometimes even "Avellaneous" or "Wood Brown"; margin involute when young. Tubes very short in young sporophores, about 4–5 mm. long in maturing ones, "Pinard Yellow" turning olivaceous with age, sinuate to subdecurrent; pores minute, 0.5 mm. (or less) across, yellow, simple, roundish at the basal portion, distinctly reticulate with yellow veins, tinged reddish at the base, equal or ventricose. Flesh straw-yellow or whitish, changing immediately to blue when exposed to the air, sometimes changing to reddish in the lower part of the stipe, rather fibrous.

Spores pale olive under the microscope, elliptical, 8–12 × 3–5 μ (9 × 4 μ).

June to Aug. 1941, from Kunming market (*5475, 5483, 7690, 7696, 7710, 7711, 7722, 7885* and *8202*).

Gilbert maintains that *B. albidus* Roq. and *B. candidus* Fr. are synonymous with *B. vitellinus* Pers. Nevertheless, there is no valuable diagnosis of the species of Persoon. It seems better to keep the name *B. albidus* Roques. The present writer is of the opinion that this species might not be a synonym of *B. radicans* Pers. ex Fr. Kallenbach's illustration is evidently different from Roques' original diagnosis. Variation of the color and the shape of the plant has been observed in the Yunnan collections. The young sporophores are usually darker in color but become much paler with age. The shape of the cap varies from convex to plano-convex with or without umbilicus. The margin of the cap is incurved when young and becomes straight with age.

BOLETUS SPECIOSUS Frost

Pileus 5–16 cm. in diameter, pulvinate becoming convex to plano-convex, dry, "Light Salmon Orange," "Capucine Orange," "Carnelian Red," "Old Rose," sometimes fading to "Onion Skin Pink," tomentose or becoming glabrous; margin entire, incurved when young, becoming straight later, usually wavy. Tubes 5–10 mm. long, "Lemon Yellow," changing to blue when bruised, uncinate to free; pores 0.5–1 mm. across, concolorous with the tubes, simple, round, becoming brownish with age. Stipe 4–11 cm. long, 1–4 cm. thick, "Eugenia Red," yellowish at the apex, "Garnet Brown" or "Brazil Red" at the base, innately reticulate with "Garnet Brown" veins throughout the stipe or only at the apex, or only streaked with "Garnet Brown" veins or fibrils, equal or attenuated toward the apex, usually dilated, central or subexcentric. Flesh solid, pale yellow, turning slowly blue-green when exposed to the air, reddish in the basal portion of the stipe.

Spores fusiform-elliptical, olivaceous under the microscope, 9–11(14) × 4–5 μ (9 × 5 μ).

July to Aug. 1941, from Kunming market, F. L. Tai et C. C. Cheo (*7732, 7746, 7737, 7740, 7757, 7751* and *7844*).

The variation of the color of this plant is rather extensive, usually from "Light Salmon Orange" to "Old Rose" through a series of intergradations. The Yunnan form has a red stipe which is sometimes yellow only at the apex (identical with the illustration in Icon. Farlow. Pl. 82). The reticulated veins on the stipe are red and very distinct but may be lost by handling or smearing. It is sometimes difficult to separate the very old specimens of this species from those of *B. sanguineus* With. merely by their external characters.

BOLETUS REGIUS Kromb.

Pileus 8 cm. in diameter, convex, dry, glabrous, pale "Pinkish Buff" to "Hydrangea Pink," becoming brown when bruised. Tubes 8 mm. long, yellow becoming greenish, uncinate; pores minute, less than 0.5 mm. in diameter, yellow, becoming greenish when bruised. Stipe 10 cm. long, 3 cm. thick, cylindrical, subbulbous at the base, yellow, reddish brown at the base, distinctly reticulate. Flesh pale yellow in the cap, whitish in the stipe, turning reddish where tunneled by worms, rather compact.

Spores pale "Cendre Green" under the microscope, fusiform-elliptical, usually 3-guttulate, 10–12(13) × 4–5 μ (11 × 4.5 μ).

June 11, 1942, W. F. Chiu, from Kunming market (*7846*).

BOLETUS SANGUINEUS With.

Pileus 7–8.5 cm. in diameter, hemispherical, dry, "Victoria Lake" to "Maroon Red," tomentose, rimose-areolate, crevices yellow; margin more or less wavy. Tubes 6 mm. long, uncinate, yellow, turning bluish green; pores roundish, 0.5–1 mm. across, concolorous with the tubes. Stipe 4.5 cm. long, 12–22 mm. thick, subequal, slightly dilated at the middle, reticulate in the lower part with dark veins and in the upper with yellow veins, yellow near the apex, "Shrimp Pink" below, brownish-black at the base. Flesh yellow, changing to blue-green.

Spores cremeous in mass, hyaline under the microscope, oblong-elliptical, 9–15 × 4–5 μ (11 × 5 μ).

Summer, 1939, Kunming market (*7732* and *7746*).

In general, the reticulation on the stipe is limited only to the upper portion, but one specimen was found with veins extending distinctly down to the base.

BOLETUS ORNATIPES Peck

Pileus 2–8 cm. in diameter, pulvinate becoming convex, dry, minutely tomentose when young, becoming glabrous with age, "Saccardo Olive" to "Olive Brown." Tubes 6–10 mm. long, "Lemon Chrome," adnate to subdecurrent; pores simple, roundish, concolorous with the tubes, unchanging. Stipe 4–11 cm. long, 1–3 cm. thick, yellow, reticulate with concolorous veins, subequal, stuffed. Flesh yellow, unchanging, rather fibrous.

Spores slightly yellowish under the microscope, elliptical to ellipsoid, 8–12 × 4–5 μ (9 × 5 μ).

June 30, 1941, C. C. Cheo, Miaokaotze, Kunming (*7729*); Sept. 14, 1941, Tapugi, C. H. Hung (*7464*); June 25, 1942, Miaokaotze, W. F. Chiu (*7861*). In coniferous woods (the dominant species of the woods is *Ketelceria Evelyniana*).

Lohwag has studied some specimens collected at Likiang, Yunnan, and found that the Likiang plant is distinct from both of the American species, *B. retipes* B. et C. and *B. ornatipes* Peck. It is said that the Likiang plant has a glabrous cap and large spores (13–

15 × 5–6 μ) (Symbolae Sinicae **II**: 57, 1937). In addition, he is of the opinion that *B. retipes* B. et C. is not synonymous with *B. ornatipes*. The present writer agrees with Lohwag in that *B. retipes* is distinct from *B. ornatipes*, but is skeptical on the validity of the new species *B. Kauffmanii*. Although the present writer has not been able to visit the Northwest of Yunnan where the type specimen of Lohwag was collected, yet in the vicinity of Kunming specimens of such type are abundant and have been collected annually. As as result of comparative studies on the characters of the plant in various stages of development, it has been found that a certain stage of growth of the plant is almost identical with Lohwag's new species. In general, the cap of this fungus is minutely tomentose when young and becomes glabrous with age, and the spores are shorter than those of Lohwag's species and those of American species, but are little broader than the latter. In Lohwag's diagnosis there is no definite description of the color of the stipe. As to flesh, Lohwag did not mention whether or not his description is based on notes of fresh specimens supplied by the collector. The color of the flesh, so far as he recorded, is "Rosaceo-griseola." Such color can be found in our dried specimens. In fresh specimens, the flesh is always yellow. The writer therefore hesitates to recognize the validity of *B. Kauffmanii* as a distinct species.

BOLETUS RIMOSELLUS Peck

Pileus 6–10 cm. in diameter, hemispherical then convex to plane, "Ochraceous Buff," "Buckthorn Brown" or "Cinnamon," maculate with brown elsewhere, subglabrous, often cracked at the margin and rimose-areolate on the surface. Tubes up to 10 mm. long, yellow, turning ochraceous with age, sinuate-free; pores minute, 2–3 per mm., concolorous with tubes. Stipe 5.5–9 cm. long, 1–2 cm. thick, "Light Pinkish Cinnamon," reticulate with brown veins, cuticle cracked and reflexed, becoming scaly, equal or tapering upward, rather thick, stuffed. Flesh white, unchanging.

Spores dark olive under the microscope, elliptical, 2–3 guttulate, 9–14 × 4.5–5.5 μ (14 × 4.5 μ).

Summer, 1938, from Kunming market (*7891*).

BOLETUS GERTRUDIAE Peck

Pileus 3–8 cm. in diameter, convex or slightly depressed, dry, glabrous, "Xanthine Orange" to "Amber Brown." Tubes 5–12 mm. long, yellow, adnate to uncinate, unchanging; pores concolorous with the tubes, about 1 mm. across, roundish. Stipe subconcolorous in the upper part and much paler in the lower part, punctato-pubescent, becoming glabrous, partially or completely reticulate with brownish veins. Flesh white, unchanging.

Spores dark olive under the microscope, oblong to subfusiform, 14–17 × 5–6 μ (15 × 5 μ).

Aug. 28, 1938, Chunhotze, Tali, C. C. Cheo (*7784*).

The reticulation of the stipe is not a constant character in this species. According to Peck's original description, the stipe is said to be smooth, but Snell reported reticulation on the stipe of his collection (Mycologia **28**: 17, 1936). The Yunnan form is rather close to Snell's collection.

BOLETUS EDULIS Bull.

Pileus 5–9 cm. in diameter, hemispherical to convex, becoming plane, dry, minutely tomentose, becoming glabrous, "Honey Yellow," "Amber Brown" to "Antique Brown," usually paler or darker than the colors described. Tubes 10–15 mm. long, white when young, becoming "Antimony Yellow," "Old Gold" or "Buff Citrine" with age, uncinate, depressed around the stem; pores 0.5–1 mm. across, concolorous with the tubes or light "Ochraceous Salmon," roundish, simple. Stipe 6–9 cm. long, 3–4 cm. thick, "Light Pinkish Buff" to "Wood Brown," distinctly reticulate with white veins, subequal, usually slightly dilated at the base or bulbous, rarely tapering downward. Flesh white, tinged pinkish in the cap.

Spores pale "Viridine Green" under the microscope, oblong-elliptical, (9)11–14(16) × 4–5 μ (12 × 5 μ).

July to Aug. 1938, from Kunming market (*5478, 5575, 5579, 5583, 7750, 7756, 7841* and *7887*); July to Aug. 1938, from Tapugi market (*7733, 7745* and *7753*); Aug. 7, 1942, Miaokaotze, Kunming, W. F. Chiu (*7855*). In coniferous woods.

The flesh of the Yunnan form is usually white and unchanging, but occasionally colored slightly pinkish. The form of the stipe is not necessarily always bulbous, but varies from slender to ventricose and bulbous, and even to tapering downward.

BOLETUS EXIMIUS Peck

Pileus 9–11 cm. in diameter, pulvinate or hemispherical becoming plane, "Hay's Maroon," "Diamine Brown" or "Hessian Brown," glabrous in the central portion, slightly tomentose at the margin. Tubes 8–14 cm. long, "Citrine," becoming ochraceous or subconcolorous with the cap, sinuate, depressed around the stem; pores 0.5–1 mm. across, "Vinaceous Purple" becoming "Russet Vinaceous," simple, roundish. Stipe 4–10 cm. long, 1–3 cm. thick, purplish, brownish in the lower portion, punctate with dark-purple or violet dots, subequal or ventricose. Flesh firm, turning lilac and punctate with dark-purple glandules in the cap, streaked with violet fibrils in the stipe.

Spores olivaceous under the microscope, narrowly elliptical to subfusiform, 11–17 × 4–5 μ (8 × 4 μ).

According to Peck's diagnosis, the tubes are said to be concolorous with the cap. In the Yunnan form, the tubes are at first citrine and then become concolorous with the cap.

**Boletus brunneissimus** sp. nov.

Pileo 3–9 cm. lato, hemisphaerico dein convexo, sicco, tomentoso, "Raw Umber," interdum rimoso-areolato; tubulis 10 mm. longis, aetate olivaceis, sinuatis v. liberis; poris minutis, 2–3 in uno mm. primitus "Carob Brown" mox "Brussels Brown" v. "Xanthine Brown" deinde "Mars Yellow," rotundis; stipite 4–9 cm. longo, 10–25 mm. crasso, "Light Pinkish Cinnamon" aetate "Nopal Yellow" v. "Chamois," punctato-striato, subaequali, deorsum attenuato; carne flava, caerulescente, stipite alba, fibrillosa, rufescente mox cyanescente; sporis olivaceis, ellipsoideis, 9–12 × 4–5 μ (11 × 5 μ).

Pileus 3–9 cm. in diameter, hemispherical then convex, dry, tomentose, "Raw Umber," darker when dry, sometimes rimose-areolate. Tubes about 1 cm. long, yellow, becoming olivaceous, sinuate or free, slightly depressed around the stipe; pores minute, about 0.5 mm. across, at first "Carob Brown" then "Brussels Brown," finally fading to "Xanthine Brown" or "Mars Yellow." Stipe 4–9 cm. long, 10–25 mm. thick, "Light Cinnamon," becoming "Nopal Yellow" or "Chamois" with age, densely covered with dark-brown-dotted elements and fibrils but smooth at the apex, subequal, occasionally tapering downward. Flesh yellow in the cap changing to blue, white and fibrous in the stipe becoming pinkish, greenish and pinkish in the basal portion of the stipe.

Spores olivaceous under the microscope, elliptical, 9–12 × 4–5 μ (11 × 5 μ).

June 21, 1941, Miaokaotze, C. C. Cheo (*7704* and *7713*); June 25, 1942, the same locality, W. F. Chiu (*7864*, **Type**); 1938 to 1941, from Kunming market (*7752, 7724, 7718, 7892* and *7899*); July 26, 1938, Mt. Omei, Szechwan, W. F. Chiu (*7687*). In mixed woods of *Ketcleeria, Pinus* and *Castanopsis*.

This plant differs from *B. umbrinus* in the yellow tubes, dark brown pores, "Raw Umber" cap and the distinctly punctate and striate stipe. In our collections, two forms have been observed: the sterile and the fertile. The sterile form possesses very short tubes usually less than 1 mm. in length, and therefore might be mistaken for a *Gyrodon*. If it were a case of immature fruiting bodies instead of sterile ones, the size and shape of the fruiting bodies would be more or less different from the mature ones. Nevertheless, there is no difference between them. Another evidence that the sterile form is really sterile instead of being immature, is the occasional tesselate crackings on the cap.

### STROBILOMYCES Scop.

**Strobilomyces glabriceps** sp. nov.

Pileo 10 cm. lato, convexo, "Carob Brown," glabro, rimoso-areolato; tubulis usque a 25 mm. longis, adnatis, decurrentibus; poris amplis, 4–5 mm. latis, alveolatis, pileo subconcolore; stipite 12 cm. longo, 15–30 mm. crasso, deorsum attenuato, basi bulboso, "Russet Brown," striato ac furfuraceo; sporis fusco-brunneis, globosis, asperulis, reticulatis, 9–12(11) $\mu$.

Pileus 10 cm. in diameter, convex, "Carob Brown," glabrous, rimose-areolate. Tubes 20–25 mm. long, decurrent; pores 4–5 mm. across, "Mikado Brown," decurrent on the stipe, angular or comb-like, rather large. Stipe 12 cm. long, 15–30 mm. thick, "Russet Brown," streaked and furfuraceous, usually tapering toward the base and slightly bulbous at the base.

Spores dark brown under the microscope, globose to subglobose, reticulate, 9–12(11) $\mu$.

June 7, 1938, Shishan, Kunming, T. K. Yien (*8034*), **Type.**

The spores of this plant are not readily distinguishable from those of *S. floccopus*, but the glabrous and rimose-areolate cap and the very decurrent tubes are distinct characters.

STROBILOMYCES FLOCCOPUS (Vahl. ex Fr.) Karsten

Pileus 2.5–8 cm. in diameter, hemispherical to convex, grayish brown, covered with dark brown, erect, recurved, or pyramidal scales, usually appendiculate on the margin. Veil membranous, grayish. Tubes 10–25 mm. long, ventricose, adnate, grayish becoming black; pores 1.5–2.5 mm. across, purplish gray turning black with age. Stipe 4–14 cm. long, 8–20 mm. thick, concolorous with the cap, fibrous-scaly. Flesh brownish to lilac in the stipe, often paler in the cap.

Spores globose to subglobose, dark brown under the microscope, reticulate, 8–11(9) $\mu$.

July to Sept. 1940, F. L. Tai, C. C. Cheo, S. T. Chao, from Kunming (*5471, 5474, 7709, 7726, 7767, 7871, 7878* and *7879*); 1938, C. C. Cheo, Tali, (*7779*); W. F. Chiu, 1938, Mt. Omei, Szechwan (*7687*). Usually in mixed woods of *Quercus variabilis* and *Pinus yunnanensis*. In one case, the present writer encountered this species under bamboo.

STROBILOMYCES RETISPORUS (Pat. et Bak.) Gilb.

Pileus 6–10 cm. in diameter, convex to plano-convex, sometimes slightly depressed at the center, dry, "Nopal Red" to "Garnet Brown," sometimes much paler and even tinged pinkish, tomentose becoming glabrous. Tubes up to 15 mm. long, "Light Dull Green Yellow," free to adnate or depressed around the stipe according to age; pores about 1 mm. across, concolorous with the tubes, roundish, compound. Stipe 7–14 cm. long, 12–20 mm. thick, "Lemon Yellow" to "Ochraceous Buff," tinged "Ochraceous Salmon" in the basal portion, reticulate with yellow veins, slightly tapering upward. Flesh "Pale Pinard Yellow," changing to reddish somewhere in the stipe especially at the base, sometimes changing to bluish somewhere in the upper part of the stipe.

Spores brown under the microscope, broad-elliptical, reticulate, 12–19 × 8–9 $\mu$ (16 × 8 $\mu$).

Aug. 7, 1942, Miaokaotze, Kunming, W. F. Chiu (*7865*); July 12, 1943, Shishan, Kunming (*8004*). In woods of *Pinus yunnanensis* and *Ketelceria Evelyniana*.

The cap of this plant varies from tomentose to glabrous while in the original diagnosis of Patouillard and Baker the cap is said to be glabrous.

## BOLETELLUS Murr.

### BOLETELLUS ANANAS (Curt.) Murr.

Pileus 2–3.5 cm. in diameter, hemispherical to convex, "Tawny Olive" with reddish brown squamules, minutely tomentose, usually rimose-areolate, becoming "Snuff Brown" and glabrous at the center; the veil formed by the extruding cuticle of the cap persistently applied to the apex of the stipe, forming a sleeve. Tubes 3–4 mm. long, "Light Cadmium Yellow," depressed around the stipe, usually changing to blue when bruised; pores 1 mm. across, concolorous with the tubes, angular, simple. Stipe 4–5 cm. long, 5–10 mm. thick, "Light Pinkish Cinnamon," "Vinaceous Cinnamon" at the base, fibrillose to subglabrous, subequal or slightly tapering upward. Flesh white, turning blue when exposed to the air.

Spores ochraceous under the microscope, elliptical to broad elliptical, longitudinally striate, 12–21 × 6–8 $\mu$ (17 × 8 $\mu$).

Sept. 15, 1938, C. C. Cheo, Chichushan, Binchwan (7805). Under *Pinus Armandii*.

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# EFFECT OF NUTRITION ON GROWTH AND MORPHOLOGY OF THE DERMATOPHYTES. 1. DEVELOPMENT OF MACROCONIDIA IN TRICHOPHYTON RUBRUM

RHODA W. BENHAM *

(WITH 2 FIGURES)

THE IDENTIFICATION OF THE DERMATOPHYTES. The dermatophytes were originally distinguished from other fungi by their pathogenicity In parasitic life they flourish only in keratin containing tissues, the skin and appendages. There are three recognized genera which were at first differentiated by the clinical picture for which each was responsible, and to some extent by their appearance in tissues. It is now recognized, however, that the morphology and biological characteristics of the fungi themselves form a sounder basis for identification.

Dermatophytes as a family show some general similarity in colony form and microscopic structure. It is, however, difficult to define them botanically. All dermatophytes produce round or pyriform microconidia often attached to the mycelium by a collarette, but hardly distinctive enough to separate the dermatophytes from other forms. The majority produce multicellular macroconidia (fuseaux) which are more distinctive.

The three genera are best defined by the character of these macroconidia (Emmons **1**). Species can be distinguished only by a combination of characteristics such as minor differences in macroconidia, differences in form and arrangement of the microconidia, and by mycelial structures known as nodular organs, spirals, "chandeliers" and "antlers." The form of colony and pigments produced are also important.

Identification of dermatophytes is often difficult because colony form, pigmentation and spore production vary greatly under dif-

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ferent conditions of culture. Some strains fail to produce on any known medium structures considered characteristic of the genus and species with which they conform in other respects. Many show minor variations from type strains which are difficult to evaluate by present methods.

INFLUENCE OF NUTRIENTS ON MORPHOLOGY. Sabouraud (2) in his monumental work, *Les Teignes*, published in 1910, emphasized the influence of culture media on growth characteristics. He devised two "proof media" and a "conservation medium" prepared from specified brands of peptone, crude glucose and crude maltose, and published exact descriptions of the gross and microscopic characteristics of numerous species when grown on these media.

Subsequent workers used these same media for identification of their cultures. When, after the first world war, the French maltose and peptone which Sabouraud used were no longer available, substitutes had to be found. Thus Weidman and Spring in 1928 (3) published a comparison of ringworm culture media. They decided that Fairchild's peptone was a fair substitute for the French peptone, though not perfect. This with crude American dextrose could be used to produce a "standard medium." About the same time, Hodges (4) compared cultures of ringworm fungi on Sabouraud's Proof medium and on media prepared with American peptones and sugars. He proposed a standard medium containing Fairchild's peptone and American granular dextrose. It was thought that if we could find a suitable standard medium readily obtainable by all workers, we would greatly advance our knowledge of these fungi. This has been more or less realized in the production of dried powders by the commercial houses. While it is advantageous to have the various workers in the field using comparable media, those now in use do not seem the final answer to the problem of increasing our understanding of these forms.

The above mentioned media were devised chiefly to obtain typical colony forms, but other factors such as pigment production and especially the microscopic morphology are more important in the identification of the dermatophytes. It has long been known that the media usually used for the isolation of fungi or for the

production of characteristic colonies, such as Sabouraud's honey or dextrose agars, are not satisfactory for the study of microscopic characteristics. Media such as corn meal, potato or wort agar are commonly used for this purpose. Realizing this inadequacy of the media in common use, Langeron (**5**) and his co-workers introduced the use of the so-called natural media, such as rice, barley and wheat grains. These were found more favorable for the production of spores.

The same medium will not, however, serve the same purpose for each species. Different species have their special requirements and preferences, *e.g.*, as shown by Lewis and Hopper (**6**), the presence of dextrose favors the production of diffusible pigment by *Microsporum canis* and *Trichophyton rubrum* but not by *Microsporum audouini* or *Trichophyton mentagrophytes*.

In the genus *Microsporum* there are three well recognized species, *M. canis*, *M. gypseum* and *M. audouini*. On all the usual media the two former species produce the characteristic macroconidia abundantly, whereas *M. audouini* forms them very rarely. An important contribution was made by Benedek (**7**) when he discovered that the growth of *M. audouini* on polished rice grains was stimulated by the presence of a bacterium which he named *B. weidmaniensis*, and that in addition to the increased growth, macroconidia characteristic of the genus *Microsporum* were formed. Conant (**8**) had previously shown that *M. audouini* would not grow on rice grains, whereas *M. canis* grew readily. These facts have been confirmed in our laboratory.

INFLUENCE OF VITAMINS. Recently, Hazen (**9**) has shown that the addition of yeast extract to rice has the same effect on *M. audouini* as inoculation with *B. weidmaniensis*, *i.e.*, it causes free growth with production of macroconidia. That vitamins may play an important part in the nutrition of fungi has been demonstrated many times. Robbins, MacKinnon and Ma (**10**) found that their strain of *Trichophyton discoides* suffered complete deficiencies for pyridoxine, i-inositol, and molecular thiamine. This organism and the faviform group in general are slow growing fungi isolated with great difficulty, and only on enriched media. This has been brought out by Fowle and Georg (**11**) in their recent report of ringworm cases contracted from cattle.

From these cases they were able to isolate *Trichophyton discoides* and *Trichophyton album* on Blood Agar Base (Difco), a heart infusion agar containing Bacto-Tryptose. This group of dermatophytes is undoubtedly one for which the media in general use are deficient to a high degree in the elements essential for their growth. Varying degrees of deficiency exist in other groups also, which, no doubt, accounts for the many variations in growth and spore production. *Microsporum audouini* is probably a deficient organism. *Trichophyton mentagrophytes,* on the other hand, has not shown any deficiencies (Burkholder **12**), and it is the one of the dermatophytes that grows most readily and produces the greatest number of characteristic structures and spores.

VARIATIONS AND MUTATIONS. One further complication in the study of these forms is the natural variations and mutations which occur, as well as the so-called pleomorphic degeneration which arises when a strain has been on artificial media for some time. These phenomena are probably influenced by the nature of the medium used. For example, Sabouraud observed that the pleomorphic degeneration is less frequent on the sugar free media. Some of the variations may be due to the presence or absence of certain growth substances or vitamins.

We find, then, that numerous characteristics on which we base our identification of dermatophytes are modified by changes in culture media. As we have previously maintained, no one culture medium is adequate for the recognition of all species. Even in a study of a single species one medium will serve one purpose only. What seems desirable is the optimum medium for the development of each significant characteristic.

THE MACROCONIDIA OF T. RUBRUM. Identification of *T. rubrum* (*purpureum*) is usually possible on the basis of its colony form on dextrose-peptone (Sabouraud) or similar agars by the elongated microconidia arising from the sides of long hyphae, and especially by the red to purple or wine-red pigment which it produces on most dextrose-containing media. The colony type varies so much, however, that there is some question as to whether the strains now called *T. rubrum* represent a single species or a group of species or variants. Even the pigment which gives the fungus its name varies in color and intensity and may occasionally fail

to appear on peptone media even if they contain dextrose. Moreover, occasionally a strain which morphologically conforms to the gypseum group develops a rose red pigment easily confused with the pigment of *T. rubrum*.

The macroconidia of *T. rubrum* were pictured by Bang (**13**) as long, narrow spores with nearly straight, parallel sides, and blunt or pointed ends, and three to ten septa. Most subsequent students of this fungus have noted these peculiar structures which are often described as pencil-shaped. Occasionally the cells of the spore are so rounded that it resembles a chain of chlamydospores. While these macrospores resemble those of the other trichophyta in having thin walls they are not easily mistaken for those produced by any other species of dermatophyte. They seem perhaps the most distinctive feature of the species *T. rubrum*. They are, however, inconstant. Rare strains with a powdery, or finely granular, or even cottony type of growth produce such spores fairly regularly on Sabouraud's honey or dextrose agar whereas they rarely are seen in more downy types. Spring (**14**) found the addition of ascitic fluid increased the frequency of their production but in our hands many strains failed to produce them on this medium.

In search for a more suitable medium, Blood Agar Base (Difco) was tried, because it had been found a good medium for the isolation and study of the faviform trichophyton group. Ten strains of *T. rubrum* were planted on this medium, and of these eight were found to produce macroconidia in good numbers, one formed only a few, and one failed to produce them. With these encouraging results it was decided to study a larger number of strains. In all, fifty strains have been studied on this and other media.

METHOD. The strains studied were for the most part isolated from patients reporting to the Vanderbilt Clinic. Included in the series also were some isolated by Hopkins and associates from army personnel stationed at Ft. Benning, Georgia. The strains were isolated on Sabouraud's honey or dextrose agar—some on potato-dextrose agar. Transfers were maintained on the dextrose agar, and transferred from this to the medium selected for study.

The cultures were identified by their appearance on potato-dextrose agar as suggested by Edgecombe (**15**), the pigment production noted and the presence or absence of macroconidia on this as well as on honey and dextrose agar was recorded. They were then transferred to Blood Agar Base (Difco) which is stated to contain the following ingredients per liter:

| | |
|---|---|
| Beef Heart, Infusion from | 500 g. |
| Bacto-Tryptose | 10 g. |
| Sodium Chloride | 5 g. |
| Bacto-Agar | 15 g. |

Twenty-three strains were also transferred to sterile moist rice grains. Examinations were made at weekly intervals and it was found that the spores usually appeared in about two weeks. Slides were prepared by teasing apart the material in a drop of sodium hydroxide and adding a cover slip. If spores were not found easily, several slides were studied to verify the results.

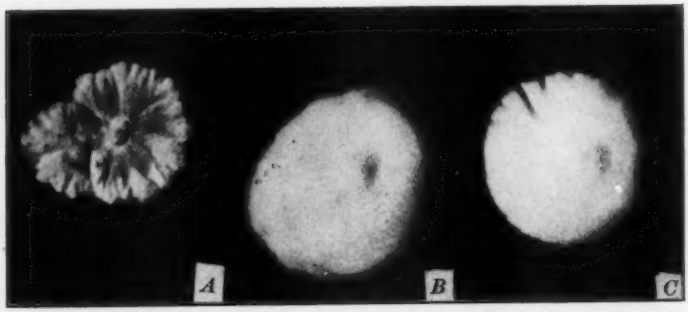


FIG. 1. Colonies of three strains of *T. rubrum* on Blood Agar Base. (A) The flat velvety type of growth. (B) The fluffy type. (C) Downy growth with flat powdery sectors at the border.

RESULTS. On the original honey or dextrose agar tubes only two of fifty strains produced macroconidia.

On rice grains a downy mycelium was visible, but as usual with this type of medium, no characteristic colony was formed. Of twenty-three strains so studied, eight were found to produce macroconidia.

On the Blood Agar Base, *Trichophyton rubrum* produces a compact and restricted type of growth. The colony may be downy,

velvety or fluffy in appearance and of about two to three cm. in diameter at the end of two weeks (FIG. 1). This growth is slow as compared with that on Sabouraud's media and the characteristic pigment is not evident. The surface may be finely wrinkled or corrugated or of the more fluffy type. Microconidia are found in great numbers. Of the fifty strains studied, all but one formed the characteristic long, slender macroconidia, on the Blood Agar Base. Twenty-three of the strains studied formed these spores in great abundance, eight in good numbers, and in seventeen only a few were seen. There was considerable variation in the length of the spores and in the number of cells, which ranged from three to ten, with the average about seven (FIG. 2). The spores form singly at the ends of hyphae or as side branches. Occasionally they were grouped (FIG. 2E). Sometimes they appear to form in the length of the hypha singly or in series and are freed by the breaking up of the hypha (FIG. 2C).

Blood Agar Base differs from Sabouraud's medium in reaction, having a pH of 6.8, in the absence of sugar and in containing beef heart infusion and "tryptose," a meat digest with a higher percentage of proteose than the usual peptones. The pH of the media did not seem to be an important factor. Macroconidia were formed as readily on the Blood Agar Base when the pH was adjusted to 5.5 or 7.4 as on the original which was 6.8.

*Results with Beef Heart Infusion.* Ten strains were grown from an agar prepared from Bacto-Beef Heart for infusions. This differed from Blood Agar Base in the omission of tryptose. This medium was not favorable for the production of macroconidia as none was found in any of the ten strains tested. Microconidia formed in great abundance.

*Results with Tryptose.* The same ten strains were inoculated on an agar containing the same amount of Bacto-Tryptose as the Blood Agar Base described above but without Beef Heart Infusion. The growth in this medium resembled that on Blood Agar Base and macroconidia were found in nine of the cultures. This would suggest that it is the Bacto-Tryptose in the Blood Agar Base which makes it so favorable for the production of macroconidia.

DISCUSSION. Forty-nine of the fifty strains tested produced

typical macroconidia on Blood Agar Base, whereas they were found in only two of the strains when grown on Sabouraud's honey or dextrose agar. It would seem that the first mentioned medium is the most favorable yet reported for the production of these

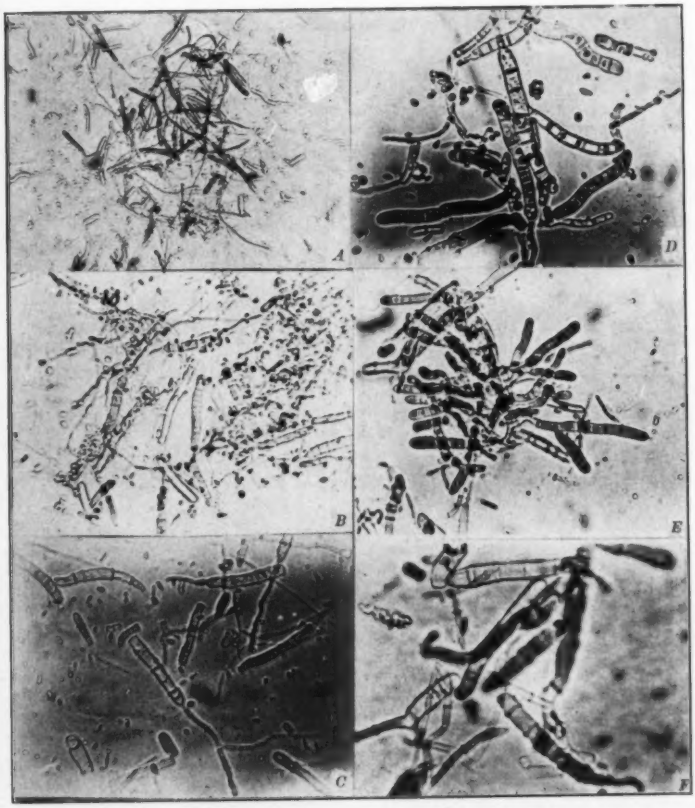


FIG. 2. Macroconidia from three strains of *T. rubrum* on Blood Agar Base. (A) × 160. (B, C, and E) × 400. (D and F) × 800.

characteristic structures. It is believed that its use will facilitate the identification of doubtful strains.

Further studies are in progress as to the relationship of proteoses or other constituents of tryptose to macroconidial formation.

SUMMARY. Of fifty strains of *Trichophyton rubrum* grown on a Heart Infusion Agar plus tryptose, known as "Blood Agar Base," only one failed to produce the characteristic macroconidia. It is believed that tryptose is the ingredient responsible for this spore production.

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# THE MEANING OF ARTICLE 57 OF THE INTERNATIONAL RULES

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Art. 57. Among Fungi with a pleomorphic life-cycle the different successive states of the same species (*anamorphoses, status*) can bear only one generic and specific name (binary), that is the earliest which has been given, starting from Fries, *Systema,* or Persoon, *Synopsis,* to the state containing the form which it has been agreed to call the perfect form, provided that the name is otherwise in accordance with the Rules. The perfect state is that which ends in the ascus stage in the *Ascomycetes,* in the basidium in the *Basidiomycetes,* in the teleutospore or its equivalent in the *Uredinales,* and in the spore in the *Ustilaginales.*

Generic and specific names given to other states have only a temporary value. They cannot replace a generic name already existing and applying to one or more species, any one of which contains the "perfect" form.

The nomenclature of Fungi which have not a pleomorphic life-cycle follows the ordinary rules.

Examples: The names *Aecidium* Pers., *Caeoma* Link, and *Uredo* Pers. designate different states (aecidiosporic with or without pseudoperidium, uredosporic) in the group *Uredinales:* the generic name *Melampsora* Cast., . . . applied to a genus which is defined by means of the teleutospores, cannot therefore be replaced by the name *Uredo* Pers. . . . since the name *Uredo* is already used to designate a state.[1]—Among the *Dothidiaceae* (*Ascomycetes*) a species of the genus *Phyllachora* Nitschke, *P. Trifolii* (Pers.) Fuck. . . . , has an older synonym, *Polythrincium Trifolii* G. Kunze . . . , based on the conidial state of this species: the name *Polythrincium* cannot displace that of *Phyllachora* because it represents an inferior state.—The name *Phoma* Fries emend. Desm. has been given to a group of *Fungi Imperfecti* (*Deuteromycetes*), several members of which have been recognized as the spermogonial state of species of the genus *Diaporthe* (*Valsaceae, Ascomycetes*): thus *Phoma Ailanthi* Sacc. belongs to *Diaporthe Ailanthi* Sacc., *Phoma alnea* (Nitschke) Sacc. to *Diaporthe alnea* Fuck., *Phoma detrusa* (Fries) Fuck. to *Diaporthe detrusa* Sacc. etc. But the perfect state of many species of the "genus" *Phoma* is not known and in some cases probably does not exist: hence the practical necessity for retaining the name *Phoma* to designate the group of *Fungi Imperfecti* in question (Briquet et al. **1935**: 17–18).

[1] The original French text, which is here the authoritative one (Briquet **1912**: vi, vii; Briquet et al. **1935**: viii), reads, ". . . le nom d'*Uredo* est déjà en usage pour désigner un état imparfait" (Briquet **1912**: 28; Briquet et al. **1935**: 45). Obviously the word "imperfect" was accidentally dropped from the English translation, which should read ". . . the name *Uredo* is already used to designate an imperfect state."

The Article here quoted is the existing law [2] relating to the nomenclature of fungi with pleomorphic life-cycle. It is an acceptable translation of the text originally adopted for Art. 49 bis (de Wildeman **1910**: 63), and as such has been operative since May, 1910. During these thirty-seven years its provisions have been faithfully followed by the majority of mycologists. There have been, however, certain proposals for the modification of its essential principle—or "interpretations" which have the same effect —and it is these modifications which are the subject of the present note.

Although there may have been earlier discussion of the matter, apparently the first formal motion for a change in the Rule was offered by Arthur (**1929**), and presented by Briquet (**1930**: 67) for action at the Cambridge congress. This motion provided for three alterations in the Rule then numbered 49 bis: (a) deletion of the reference to the works of Persoon and Fries as starting-points; (b) an addition to the sentence designating perfect states, so as to have it read, "The perfect state is that which ends . . . in the uredospore or the teleutospore (sporophyte) in the Uredinales"; and (c) a complete replacement of the first sentence under "Examples" by the sentences: "The names *Aecidium* Pers., *Roestelia* Reb., *Accidiolum* Unger, and *Peridermium* Chev. designate different states of the gametophyte in the group *Uredinales*. The generic name *Aecidium* Pers. . . ., belonging to the gametophytic state, cannot displace *Gymnosporangium* Hedw. f. . . ., based upon the sporophyte." The essence of this last proposed change is to substitute in two places examples proscribing nomenclature based on the aecial [3] state for examples proscribing the uredinial.

This motion was discussed by its author and others at a meeting of the subsection of botanical nomenclature at the Cambridge congress; no action was taken (Brooks & Chipp **1931**: 598–9). The following day it was announced that Maire and Arthur "were agreed in proposing that Art. 49 bis be maintained until the next

[2] But cf. footnote 1.

[3] The terminology here employed by the writer for rust-spores and -sori is that proposed as standard by Arthur (**1905**); in quotations the terminology is of course that of the author quoted.

congress" (Brooks & Chipp **1931**: 622). There was some further discussion, but no further action, concerning Arthur's motion; by vote of the subsection (Brooks & Chipp **1931**: 623) Art. 49 bis was retained and was referred to the editorial committee for review of the wording. The third edition of the International Rules constitutes the report of that committee; as was proper, in view of the vote, no essential alteration was made in the Rule (Briquet et al. **1935**: 44, 17). Arthur subsequently declared (**1934**: 474), "No change in the Rule is advocated. The Rule is to stand as it is." The amendment under discussion was not presented at the following congress (Sprague **1935**), and unless it has been renewed in some place that has escaped notice, is not now an issue.

In 1934 Arthur published an "Interpretation of Rule 49 bis." His five pages of discussion, which should be read by anyone interested in the subject, are too long for quotation *in extensu*, and will have to be dealt with in excerpt or summary. The major thesis, as stated in the author's summary, is that "the Rule [quoted at the beginning of this note] excludes aecidiosporic names (of the gametophytic state), but includes both uredosporic and teleutosporic names (of the sporophytic, or 'perfect' state)" (Arthur **1934**: 476). This "interpretation," like the motion for amendment which it replaced, would, of course, legitimate much of the nomenclature adopted by its author in his American-Code days. It is supported by an ingenious argument based on the cytological evolution of the rusts:

> There are two *states* in every species of this order whatever its generic connection, the gametophytic or haploid, which bears aecidiospores, and the sporophytic or diploid, which bears uredospores and teliospores. . . . In reduced species, especially the so-called short-cycle species, the two states are much curtailed, and some of the spore-forms may be suppressed. . . .
>
> The rule clearly sets forth that "the perfect state is that which ends in the teleutospore." . . . Neither the wording of the rule nor the history of spore development excludes the uredospore, a product of the sporophyte, and a normal part of the "perfect state," although the aecidiospore, a product of the gametophyte, is clearly excluded.
>
> . . . Every permanent generic or specific name must be founded upon the "perfect state," which is equivalent to saying that it must be founded upon the sporophytic state, a state which bears uredospores and teleutospores.
>
> Uredospores . . . are borne on sporophytic mycelium and are binucleate (Arthur **1934**: 472–3).

Leaving aside the debatable and much debated implication that dikaryotic cells are diploid and sporophytic, one may summarize Arthur's argument as follows: (a) The uninucleate and the binucleate phases of the life-cycle of rusts are the "states" referred to by the Rules. (b) The "imperfect state" consists of the haploid, or uninucleate, mycelium and the spores that it bears; the "perfect state" consists of the binucleate mycelium and the spores that it bears. (c) Uredospores and teliospores are borne on binucleate mycelium and therefore both belong to the perfect state; pycniospores and aeciospores are borne on uninucleate mycelium and therefore both belong to the imperfect state. (d) Names applied to pycnial or aecial stages are hence only "temporary names," whereas names applied to uredinial and telial stages are, equally, "permanent names."

Whether one is acquainted with the life-cycle of rusts only as exemplified in the discussions of *Puccinia graminis* in elementary textbooks, or has followed the very considerable number of cytological papers that have appeared in the literature beginning with the later 1800's, one must be at a loss to understand the quoted phrases "gametophytic or haploid, which bears aecidiospores," "the aecidiospore, a product of the gametophyte," "aecidiosporic names (of the gametophytic state)," or the further note, "It has been found desirable to exclude aecidiosporic names, or any others applied to the gametophyte . . ." (Arthur **1934**: 475). For all aeciospores, like all uredospores and teliospores (exception being made for rare abnormalities), are binucleate, and there appears to be no record of a dikaryotic spore borne on a haploid mycelium. Beginning at least as early as 1896 (Sappin-Trouffy **1896**: 77, 219, et passim; fig. 4, 5, 14, 34), aeciospores have regularly been shown to be abstricted from the ends of binucleate hyphae; Arthur's *Plant Rusts* (written with the collaboration of Kern, Orton, Fromme, Jackson, Mains, and Bisby) represents aeciospores as belonging to the "sporophyte or diplophase," along with at least most of the mycelium which bears them (Arthur et al. **1929**: fig. 59); Jackson states (**1931**: 16; cf. also 15–17) that "aeciospores, urediniospores, and teliospores are diploid, and the change from the haploid to the diploid phase takes place ordinarily in the primordium of the initial sorus, regardless of the type of spore to follow." There is

no need to burden the argument with further evidence, which can be cited *ad libitum* and *ad nauseam*.

It is, as earlier stated, difficult to understand such statements as occur, in the works of various uredinologists, assigning aeciospores to the "gametophytic," or haploid, or uninucleate phase. It is true that the aecial primordium is normally formed of uninucleate, or at least non-dikaryotic, cells, and that a part of the cells of the mature aecium may remain so. But the same thing is true, *mutatis mutandis*, of all ascocarps—the fructification is composed of an outer sterile layer of uninucleate mycelium and an enclosed system, of greater or less extent and complexity, of so-called "sporophytic" mycelium, on which are borne the organs of fructification. The asci are not on that account "gametophytic," nor is an ascigerous fructification imperfect. Perhaps the statements referred to, that "the aecidiospore [is] a product of the gametophyte," are to be understood not as literal statements of cytological truth, but as merely conventional, as short-hand attempts to convey that the aecium is usually a mixed fructification—*i.e.*, is usually made up of both mono- and dikaryotic hyphae. The aecium is probably such whether the dikaryotic phase is limited to fusion cells, the short hyphae which develop from them, and aeciospores, as supposed by the earlier workers (*e.g.*, Blackman **1904**: 338), or whether there is extensive dikaryotization both within the aecium and of the peripheral mycelium (Allen **1935**: 1058–60, and pl. 7, fig. f, h; Brown **1935**). There is, however, a not inconsiderable number of rusts in which the telium is likewise a mixed fructification, and others occur where uredinia are made up of both kinds of mycelium (Jackson **1931**: 18, 74). Are these telia and uredinia on that account to be called gametophytic? Not many mycologists have thought so. The telia and teliospores of "micro" rusts are quite as perfect, to most students of the fungi, as those of long-cycle species even though they develop at that stage which in long-cycle rusts is occupied by aecia and aeciospores. If development on binucleate mycelium be the criterion of the perfect state, then aeciospores, uredospores, and teliospores are equally perfect, and names based on any of these stages are of equal nomenclatorial standing; if development in a cytologically mixed fructification be the criterion of the imperfect state, then

numbers of rusts forming teliospores are quite as imperfect as those which so far as known form only pycnio- and aeciospores.

So much for the interpretation that "state" means cytological phase. It may be thought that this is a strained "interpretation" of the Rule. It is, nevertheless, one which the text can probably be made to bear. The examples, though not so explicit as could be wished, probably imply a more reasonable meaning. "The names *Aecidium* Pers., *Caeoma* Link, and *Uredo* Pers. designate different states (aecidiosporic with or without pseudoperidium, uredosporic) in the group Uredinales." Not haploid and diploid states, nor uni- and binucleate states, nor gametophytic and sporophytic states, but "aecidiosporic" and "uredosporic" states. So much makes clear one matter: the word "state" in the text of the Rule does not refer to, and is not synonymous with, "cytological phase." Rather it refers either to what is often called a spore-form ("aecidiosporic, uredosporic"), or else, more precisely, to a fructification bearing or containing reproductive structures of a particular sort. Further reading of the examples will confirm this: *"Polythrincium Trifolii* . . . [is] based on the conidial state of [*Phyllachora Trifolii*]"; "the spermogonial state of species of the genus *Diaporthe*." Of these further examples the first ("conidial state") could refer to either a spore-type or a fructification; but the second ("spermogonial state") can refer only to a fructification: a spermogonium is not a kind of spore. Reading of the rest of the Rule itself, finally, would seem to settle the question: "The perfect state is that which ends in the ascus stage in the *Ascomycetes,* in the basidium in the *Basidiomycetes,* in the teleutospore or its equivalent in the *Uredinales,* and in the spore in the *Ustilaginales.*" Since only teliospore and [smut] spore are in any sense spores, while both they and ascus and basidium are reproductive bodies, the one meaning of "state" that makes sense with the whole text of the Rule and with all the examples is "fructification"; and the reference to the rusts must be understood to say, "The perfect *fructification* is that which ends in the teleutospore or its equivalent in the *Uredinales.*" [4]

[4] Although not provided for by Art. 57, it is quite consistent with that Rule to speak of assimilative states, such as (nonsporiferous) mycelial, or plasmodial.

And finally, the decisive pronouncement of the Rules on this matter is the one already quoted from the authoritative (French) text (see footnote 1, p. 241): "the name *Uredo* is already used to designate an imperfect state." Even though it be admitted that there is a possible confusion between *Uredo* the form genus and a uredo—*i.e.*, the uredinial stage—still it must also be admitted that *Uredo* is rejected by the Rules as an alternative to *Melampsora* not because it is the name of a form genus but because it is used to designate an imperfect state; and it must be admitted further that by inevitable implication the uredo (uncapitalized) is thereby declared to belong to the imperfect state. So far as the Rules are concerned, then, the matter is definitely settled.

The reason for here attempting a solution of the problem raised concerning the uredinial state is not a lively concern for the nomenclature of the Uredinales.[5] The principle involved extends far beyond that order. Arthur seems not to have been aware of this, for he wrote "Rule 49 bis applies solely to the Ascomycetes and Uredinales" (Arthur **1934**: 471). The origin of this misapprehension is not apparent; certainly it cannot be the text of the Article itself, for that begins with the phrase "among Fungi with a pleomorphic life-cycle"—that is, among at least a considerable number of fungi of every class without exception (even the Myxomycetes having had binomials applied to the plasmodial state which except for the operation of this article have the same standing as binomials applied to the sporangial state). Furthermore the Article at least indicates, and probably defines, the perfect state

[5] Except for the inevitable corollary that it validates the nomenclature applied to aecial fructifications, Arthur's "interpretation" is of perhaps only minor importance for the rusts. In 1930 he stated that "if . . . the uredosporic state were not placed on the same footing as the teleutosporic state, a great number of specific names would be invalidated and there would result numerous changes"—seventy or eighty out of a thousand species that he had reviewed (Brooks & Chipp **1931**: 598–9). By 1934 the situation had bettered itself, for Arthur was able to state, "The only recent work embracing all known species of the Uredinales from every part of the world is that of Sydows' Monographia Uredinearum. The work is accurately compiled, and the synonymy is essentially complete. The names of species recognized are with rare exceptions those which are in general use. It is interesting to note that out of 2333 species embraced in this work having teleutospores only 26 species are affected by the varying opinion regarding the uredospore . . ." (Arthur **1934**: 474).

not only for Ascomyctes and rusts, but also for the Ustilaginales and [other] Basidiomycetes. It is among the Basidiomycetes not included in the Uredinales that the "interpretation" that the perfect state includes all fructifications of the binucleate mycelium would give most trouble. Arthur was under misapprehension here also, since he wrote, "Uredospores . . . are borne on sporophytic mycelium and are binucleate, a condition which is not generally true of conidia as ordinarily considered. . . . True conidia, as in the Ascomycetes and higher Basidiomycetes, are borne on gametophytic mycelium and are haploid" (**1934**: 473–4). Now conidia borne on the basidial fructification, and therefore presumably on binucleate mycelium, were described at least as long ago as 1853 (Tulasne **1853**: 197, 216–219); they were reported by Patouillard in 1887 (p. 56–67), by Brefeld in 1889 (p. 10; pl. 1, fig. 11), and since then by a considerable number of authors. Lyman, for example, published figures and descriptions of conidia borne on the binucleate mycelium of a number of Basidiomycetes (**1907**: 168, pl. 20, fig. 52–3; 174, pl. 20, fig. 69; 159; 178; 186, pl. 22, fig. 116–25 and pl. 25, fig. 134). Summaries of conidial forms—including dikaryotic ones—reported in Basidiomycetes have recently been given by Martens & Vandendries (**1933**: 345–52) and by Biggs (**1938**: 66–7). A number of these conidial states have of course never been given names, and are therefore of no nomenclatorial importance. The situation is different for the named conidial states.

*Monilia candicans* Sacc. 1876 is a conidial state recently found by Linder (**1942**) to be congeneric with the type of *Oidium*, and accordingly redescribed and transferred to that genus. It is the conidial fructification of *Corticium pruinatum* Bres. 1903 (= *Pellicularia pruinata*) (Rogers **1943**). Since both conidiophores and basidia arise from the same mycelium, under the concept of a "state" as a cytologic phase Saccardo's epithet "candicans," which long antedates Bresadola's, must replace the latter in either *Corticium* or *Pellicularia*. *Hymenochaete tomentosa* B. & C. 1868 was found by Linder to be no *Hymenochaete*, but similarly a conidial state, and accordingly was renamed *Oidium tomentosum* (Linder **1942**); in it also the conidiophores arise from the same repent hyphae which bear the subhymenial hyphae of a *Pellicularia*.

The latter was recently described (Rogers **1943**) as *P. lembospora;* but if its conidial state is held to belong to the perfect state, the specific epithet must be "tomentosum." *Oidium Morgani* Linder 1942, *O. effusum* (B. & C. 1875) Linder, and *O. pulveraceum* (Ellis 1884) Linder have clamp connections on the conidiophores and therefore could also be considered to belong to the perfect state. At present it would be as difficult to place them in their correct basidomycetous genera as it would be to decide whether the unconnected uredinial state of a rust should be assigned to *Puccinia* or *Uromyces* or to any of a number of other genera which do not have distinctive uredinial fructifications; but if ever they are connected with a basidial fructification, *O. effusum* and *O. pulveraceum,* because of their early date, might cause nomenclatorial trouble.

Other examples could be cited; but so much for the nomenclature of species. *Rhizoctonia Crocorum,* the type of *Rhizoctonia* Fries 1822, has been shown by Buddin & Wakefield (**1927**: 125, 134) to be binucleate and to be connected with *Helicobasidium purpureum,* the type of *Helicobasidium* Pat. 1887. Under the interpretation that the binucleate phase is the perfect state, the name *Rhizoctonia* would replace *Helicobasidium,* and a new name would have to be found for the unconnected species now collected in *Rhizoctonia. Aegerita candida,* the type of the genus *Aegerita* Pers. ex Fr. 1822, has been shown by Lyman (**1907**: 167-172) and by von Höhnel & Litschauer (**1907**: 810–815) to be connected with a *Peniophora* (*P. candida* Lyman, = *P. Aegerita* H. & L.). The *Aegerita* consists of bulbil-like aggregates of cells, said to fragment into conidia, but when formed connected by well defined clamps. It could then be considered to belong to the perfect stage. If it does, then *Aegerita* is a genus not of the Fungi Imperfecti but of the Basidiomycetes, and must replace *Peniophora* Cooke 1879. One more case: *Michenera artocreas* is the type of the genus *Michenera* B. & C. 1868; it is a cupulate conidial fructification associated with *Aleurodiscus subgiganteus.* Since it sometimes springs from the hymenium of the latter basidiomycete (Lyman **1907**: 159), it must be supposed to be binucleate. If all binucleate fructifications are perfect, the name *Michenera* must replace *Aleurodiscus* Rab. ex Cke. 1875.

These examples are taken from the Thelephoraceae not because similar ones could not be found elsewhere, but because that family is most familiar to the writer. The genus *Rhizomorpha* Pers. ex Gray 1821 from the beginning included a number of species, but the most surely recognizable and best known is *R. subcorticalis*, and that evidently must be the type of the genus. That name is based on the familiar shoe-strings (Hartig **1878**: 59–62) which develop before and with the sporophores of *Armillaria mellea* (Vahl ex Fr.) Quél. 1872 (or *Armillariella mellea* (Vahl ex Fr.) Karst. 1881). Since the rhizomorphs give rise to the basidiocarps, presumably both structures are dikaryotic, and would belong to the perfect state—in which case the name *Armillaria* (or, if *Agaricus melleus* does not belong to that genus, at any rate *Armillariella*) must be replaced by *Rhizomorpha*. The genus *Rhacophyllus* Berk. & Br. 1871, based on *R. lilacinus*, has agaric-like fructifications bearing instead of gills rows of bulbils (or better, perhaps, peridioles) under the pileus (cf. Petch **1926**: 238). The basidiophorous stage is not known, nor even whether the form should be accounted an imperfect agaric or an imperfect gasteromycete. But it has been shown to be dikaryotic, and may be, as suggested by Patouillard (**1913**: 220), a stage of *Psathyrella disseminata* (Pers. ex Fr.) Gillet 1878, which is *Pseudocoprinus disseminatus* (Pers. ex Fr.) Kühner 1928. If it proved to be so, under Arthur's interpretation *Rhacophyllus* would displace one or both of those generic names.

Faull's discussion (**1932**: 5–11) of the particular problem of *Milesia* vs. *Milesina*, which Arthur (**1933**) thought significant in this connection, is actually not pertinent here. Faull's contention is that although the description of *Milesia* applies to only the uredinial stage that name ought to stand against the later *Milesina*, based on the telial stage. But on his showing "some of [White's] type materials [of *Milesia Polygoni* White = *M. Polypodii* White] are teliosporic" (Faull **1932**: 8, 23), and therefore White's names are in fact "the earliest which [have] been given . . . to the state containing the form which it has been agreed to call the perfect form, . . . that which ends in . . . the teliospore" (Briquet et al. **1935**: 17). The fact that "White's definition of both the genus and the species applied to the uredinial phase only" (Faull **1932**: 5) affects

the nomenclatorial status of the genus and species no more than does any other faulty original description—that is, not in the least. The law on this point is contained in Art. 18: "The application of names of taxonomic groups is determined by means of *nomenclatorial types*. . . . The type . . . of a generic name is a species, that of the name of a species . . . is usually a specimen . . ." (Briquet et al. **1935**: 3). The opposing doctrine (that an inaccurate description rather than a good specimen determines the character of a species) would mean that most early names of fungi would have to be abandoned—*e.g.*, *Agaricus* Fr., described as "ascigerous" (Fries **1821**: 8). *Milesia* is then the valid name—if all of Faull's statements of fact be accepted as correct—under the Rules.

As earlier noted, Arthur stated in 1934 "that no change in the Rule is advocated." The agenda for the 1935 congress included, nevertheless, a motion over his name for modification of Art. 57, the former Art. 49 bis (Sprague **1935**: 42). It is composed of two parts: the first is to substitute "Linnaeus, *Species Plantarum*" for "Persoon, *Synopsis*." This is merely a corollary of a motion printed elsewhere to move the starting-point for rusts back from 1801 to 1753, and does not affect the "interpretation" of the provisions concerning pleomorphic fungi. The second part is a proposal to delete all that part of the "Examples" having to do with rusts and to substitute the statement, "The generic names *Aecidium, Roestelia, Peridermium,* and *Uredo* not only are the names of genera, but are also employed to designate different stages in the group *Uredinales*." As an "example" this appears somewhat pointless; it is a Rule, if anything; and it certainly is not a mere revision of the sentence it replaces, but a quite different statement on quite a different subject. Whatever the intention of the proposed amendment, its effect is to change the designation of the uredinial and the several aecial fructifications from "states" to "stages." To that extent it constitutes a change not only in the "Examples" but in Art. 57 itself, and should in the interest of consistency be rejected by those who are not prepared to follow consistently and to its conclusion Arthur's interpretation of the term "state" and of Art. 57. Even the adoption of the amendment would, however, not constitute unassailable endorsement of that

interpretation, since in the examples taken from the Ascomycetes the state would still be "conidial" or "spermogonial"; but it would render equivocal what is now unequivocal. At the Amsterdam congress (Sirks **1936**: 368) the appointment of a special committee to study this amendment was authorized, and presumably the matter will come up at the next congress.

Bisby (**1944**: 283) has recently offered for discussion a complete revision of Art. 57. His version has much to commend it; it would settle authoritatively a number of points where now one must be guided by inference or custom. It is, however, somewhat less explicit than the existent Rule; in particular, it does not provide any means, as Art. 57 has been shown to do, for determining in what sense "state" can legitimately be used. And like Arthur's proposed amendment, it declares that "the perfect state . . . in Uredinales [is] the Uredo or telial stage." It would seem preferable to insert its provisions concerning type specimens, the citation of authorities, and, if such a provision is desirable, concerning precedence among imperfect states, in their several proper places in the text of the Rules, and to the writer at least, to leave Art. 57 quite, or at any rate in essence, unchanged.

For, as must appear from the present discussion, the "states" referred to by the Rules are not cytological phases, but organs of fructification (or assimilation); and the uredinial state has no greater claim to recognition as a perfect state than has the aecial—or, in various other Basidiomycetes, conidial, sclerotial, and mycelial states. Furthermore, it is illogical (unless Art. 57 is to be abolished utterly), it is contrary to the practise of the great majority of mycologists, and it cannot but be confusing, to treat two or more different states in the same life-cycle as equally perfect, and the names based on them as of equal value in determining the legitimate name of a fungus. Furthermore, the assignment to the uredinial fructification of "perfect" status can apparently be accomplished only on the basis of a principle which would radically and extensively alter the nomenclature not only of the Uredinales (since it would validate names applied to the aecial fructification), but of other and larger groups of Basidiomycetes. And finally, it is proper to take into account that the concept of perfect and imperfect states is not strictly a nomenclatorial one, not even merely

a taxonomic one, but is as well a distinction important in fungus morphology. One who values precision in language, who values language as an instrument for developing and conveying ideas, can scarcely accept with unconcern the debasement of one of the special tools of his trade, the deliberate creation of an ambiguity which renders valueless a single one of the terminological distinctions with which he works.

In this discussion the writer has had the benefit of discussion or comment by Dr. G. R. Bisby, Dr. G. B. Cummins, Dr. B. O. Dodge, Dr. H. S. Jackson, and Dr. G. W. Martin, none of whom, however, is in any degree thereby committed to the conclusions expressed.

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# VARIATION IN MONTAGNITES ARENARIUS (DC.)

ELIZABETH EATON MORSE

(WITH 19 FIGURES)

*Montagnites* is a peculiar fungus which occurs in arid regions in the Western United States. It has been collected on the plains of eastern Oregon, on California deserts, in New Mexico, in the region of San Diego and on the peninsula of California. Its peculiarity lies in the fact that whereas it arises as a two-layered, hypogaeic gasteromycete, in passing through its various stages of development, it approaches at maturity the family Agaricaceae, resembling most the genus *Coprinus*. It has a cap and stem, also a volva such as is met in some coprini (**3**, p. 206 f. 108). It has on the sides of the gills the large, vesiculose cystidia of *Coprinus*, also black spores. However, it is removed from *Coprinus* by the structure of the gills and by their weak attachment to the cap; furthermore, *Montagnites* never deliquesces.

It thus appears that *Montagnites* represents a transition between two large groups of fungi, gasteromycetes and agarics; it is placed by Clements and Shear in the family Agaricaceae in their Genera of Fungi, p. 230, and by Killerman (**5**, p. 230).

Fries discovered that he and De Candolle were applying the genus name *Montagnea* to two groups of plants far removed, De Candolle (**2**) to a member of the composite family and Fries to the above fungus. Fries, therefore, decided to change the ending of *Montagnea* just a little ("paullulum"), which resulted in *Montagnites;* he honored his contemporary by appropriating his name for the species name, explaining the situation as follows: "Primo dixi *Montagneam,* sed quum Decandolle eodem anno proposuerit novum genus sub eodem nomine, meum paullulum mutavi." (See Fries, Hymenomycetes Eur., p. 319, 1874.) Fries published *Montagnea* in Genera Hymenomycetum (p. 7) in April, 1836; De Candolle published *Montagnea* in October, 1836. Fries' claim to *Montagnea* cannot be disputed, but he decided to use *Montagnites.*

*Montagnites candollei* was promptly adopted in all the literature, and it seems to me ill advised to try to revive the earlier use of the name *Montagnea* (Mycologia **35**, p. 450, 1943). Fries' explanation for its discontinuance should be adequate.[1]

It must be admitted that Fries' proposal of *candollei* as a species name is out of order, since two other names had already been given to this fungus—*Agaricus radiosus* Pallas, 1777 (see Hollos, 1904) and *Agaricus arenarius* DC. (see Fries, Epicrisis, p. 241, 1838).

There is extraordinary variation in size, recognized by the French mycologist Patouillard (**7**, p. 219) in erecting the species *Montagnites tenuis,* characterized also by small spores. Cleland accepts wide variations in spore size in a single species (**1**, p. 164). Long finds very large and very small specimens growing in close proximity, but advises against even a use of variety.

Hollos (**4**, 30–33, pl. 1–2) shows in his illustrations numerous rhizomorphs issuing from volvae. We have not observed such in any of the specimens acquired by us. Doubtless they were present but lost in collecting.

Description of **Montagnites arenarius** (DC.) comb. nov.

*Agaricus radiosus* Pallas Reise **2**: 744. pl. 4, f. 3. 1777.
*Agaricus arenarius* DC. Fl. Franç., tome **5**: (vol. 6): 45. 1815.
*Montagnites candollei* Fr. Epicrisis, p. 241. 1838.
*Montagnites pallasii* Fr. Epicrisis, p. 241. 1838.
*Montagnea candollei* Fr. ap. Corda Icon. Fung. **6** (edit. Zobel): 85. pl. 20, f. 146. 1854.
*Montagnites radiosus* (Pall.) P. Henn. Hedw. Beibl. **40**: (98). 1901.
*Montagnites radiosus* (Pall.) Hollos Gasterom. Ungarns, p. 30. pl. 1, f. 16–23; pl. 2, f. 1–4. 1904.
*Montagnea arenaria* (DC.) Zeller, Mycologia **35**: 418. 1943 (as "arenarius").

I can do no better than refer readers to the detailed description of this fungus by Doctor John Burton Cleland in "Toadstools and Mushrooms" (**1**: 162). A condensed statement made from that description follows:

[1] Conservation of Name *Montagnites.*
In consideration of the almost universal acceptance and endorsement of the name *Montagnites* Fries, I hereby request the next Congress to consider placing it on the list of Nomina Conservanda.

(After Montagne, the French mycologist). Universal veil forming a volva, persistent. Stem dilated at the apex into an orbicular disc smooth on both sides, to the margin of which the free gills are attached. Gills radiating, sickle-shaped, persistent, with obtuse edges, without an enveloping cuticle. Trama cellular. Spores oblong, smooth, black-fuscous, basidia tetrasporous.

Pileus 1.2–2.5 cm., occasionally more, at first deeply inturned below towards the stem, "tucked in," covered by the grayish to dirty white universal veil through which shows the ribbing of the gills. Gills to 3 mm. wide, very close like the leaves of a book, attached along the periphery of the disc, at first covered by the delicate universal veil, surface slightly wrinkled, carbonaceous; on old plants the gills expand outwards and become ragged.

Stem 2.5–7.6 cm. long, 3–7 mm. thick, equal or attenuated upwards, or slightly so downwards.

Cleland records great variation in the shape of spores: "spherical," "irregularly spherical," "elliptical," "triangular," "ovate"; he records spore measurements in very many collections: Mount Elba, 19–24 × 11–12.8 μ (rarely 27 × 21 μ); Woodford Creek, 15–26 × 9–15 μ; Encounter Bay, (with stem 2 mm. thick) small spores, 7.5 × 4.4 μ, which are elliptical to irregular or almost triangular. It thus seems, "as suggested by others," so states Cleland, "that we are dealing with a single species, highly variable as regards its size and robustness and the size and shape of the spores" (**1**, p. 164). Lloyd (**6**, p. 1165) also comments briefly on the extreme variation of this species.

## SUMMARY

Greatest stature in Central Europe, Hollós. (28 cm.)

Greatest robustness in New Mexico Coll. Long. Cap 5½ cm. wide, stem to 1½ cm. wide.

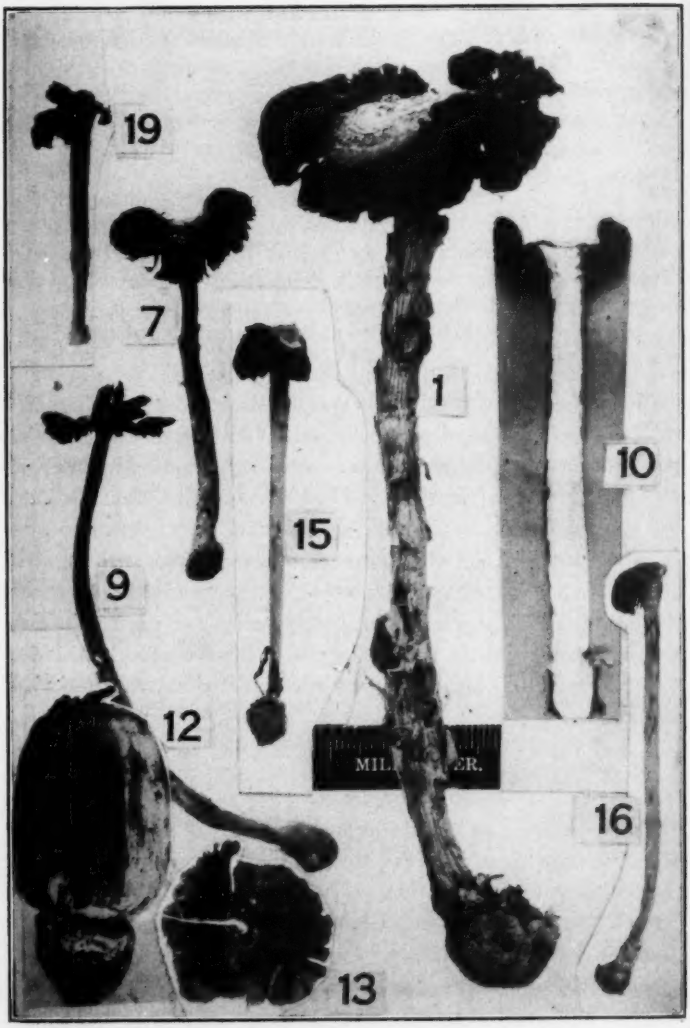
Most diminutive, Galapagos Islands on the equator, Howell.

Darkest scales, Peninsula of Lower California, Mexico, Wiggins and McMurphy.

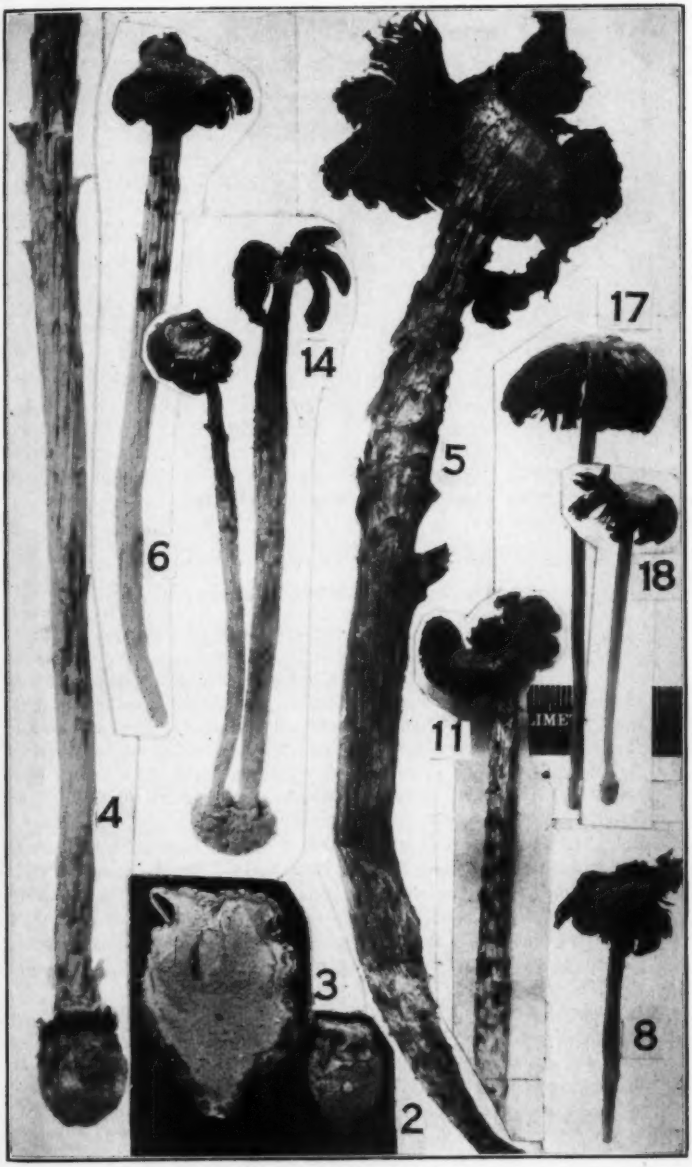
Largest and smallest spores, South Australia, Cleland.

Greatest variability in shape of spores, Central Europe, Hollós.

Narrowest stem, Encounter Bay, 2 mm. thick, Cleland.




*Montagnites arenarius.*




*Montagnites arenarius.*

ACKNOWLEDGMENTS

I wish to extend grateful thanks to those who have supplied material making it possible to publish these illustrations; also to my editors and all those who have assisted in many ways.

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## EXPLANATIONS AND DESCRIPTIONS

Photographs by W. C. Matthews

*Montagnites arenarius.* FIG. 1. A superior example of a mature specimen, 18 cm. tall, head, stem and volva intact; disc roughened by remnants of universal veil; lamellae crowded, blackened by ripening spores, attached to margin of disc, showing tendency to roll back on the disc; stem equal, cuticle split longitudinally and transversely, broken into coarse scales with tips pointing upwards; volva globose, scaly, sanded, showing ligulate scales issuing at summit of volva; spores 10–12 × 6–7 μ. Dry Lake, near Lancaster, California, Mojave Desert, April 4, 1932. Collector, Harold E. Bailey.

2,—Volva, 12 mm. tall, globose, wrinkled in drying, blunt-pointed, sanded, region Albuquerque, New Mexico, June 30, 1937, W. H. Long. (× 1)

3,—Vertical median section, shows 2-layered peridium, spongy basal tissue, plant in embryo discernible. (× 2)

4,—Incomplete stem and volva of a "giant" specimen, woody, deeply ridged, slightly twisted, scales pointing up, orifices often within scales ("chimneys?").

5,—A "giant" specimen, over-mature, stem coarse-scaly, ventricose, widened at summit into a circular disc to the margin of which lamellae, tattered and torn, are attached by a thread, some plates missing; stem narrowed to base, volva lost.

6,—Specimen of medium stature, upper half of stem scaly, volva missing.

7,—A complete specimen, dwarfed.

8,—A diminutive specimen, volva lost. FIGS. 2–8. Preserved in U. S. Department of Agriculture, Beltsville, also in the herbarium of the University of California, Berkeley, and in the Long herbarium, Albuquerque, New Mexico.

9,—Specimen complete, stem long, slender, curved.

10,—Vertical, median section, central tissues somewhat floccose, volva close fitting.

11,—Stem, dark-scaled, scales closely adherent, spores 12–16.8 × 10.8 μ. Old, dried specimens from Hamilton Ranch to the Cape, Peninsula Lower California, April 28, 1931, Wiggins and McMurphy. Bank of Salinas River, ranch, Harry Hatch, Templeton, Calif., San Luis Obispo Co., April 2, 1932; another collection, pileus 18 mm. broad, gills extending to 30 mm., making pileus seem that broad; stipe 10–16 cm. tall, 7–8 mm. broad, half buried in sand, Ira L. Wiggins.

12,—Var. *texensis* Berk. and Curt., young plant grown in laboratory, with typical volva, remnants of universal veil on top of cap, cap closed down over stipe which elongates rapidly.

13,—Underside of a mature cap, broken from hollow stem, showing a weft of tissue, free, floccose. FIGS. 12 and 13, laboratory, Riverside, Calif., Oct. 1939, C. O. Smith. (See Madroño, Vol. 5, No. 4, pp. 119.)

14,—Two individuals, each volvate, ensconced in *one* volva, herbarium, Univ. Calif., Berkeley, May 1, 1914, T. S. and K. L. Brandegee.

15, 16,—Two slender specimens, volvate, long, ligulate scales issuing from top of volva. 15; collected from arid region eastern Oregon, donated herbarium Univ. Calif., 1944, S. M. Zeller.

17, 18, 19,—Small specimens, volvae intact, stems slender, fairly equal, collected off Ecuador, on South Seymour Island, Templeton Crocker Expedition, June 10, 1932, John Thomas Howell; from Miss Alice Eastwood, Academy of Sciences, San Francisco, Calif.

# NEW GENERA OF FUNGI–IV

R. Singer

## 1. The Genus Neopaxillus Sing.

A Brazilian agaric collected by J. Rick [1] and now preserved at the Farlow Herbarium exhibits characters that do not fit into any known genus. It is, in certain regards, intermediate between the Cortinariaceae (*Cortinarius*, etc.) and the Paxillaceae (*Linderomyces, Paxillus*), having the spore characters more nearly of a *Cortinarius* (though the ornamentation is not identical with that of any *Cortinarius* known to the author), and the habit and trama of the Paxillaceae. The author considers this collection as the type of a new genus and species.

**Neopaxillus** Sing. gen. nov.

Habitu *Phylloporum* in mentem revocante; lamellis distantibus, decurrentibus; tramate hymenophorali e mediostrato axillari et hymenopodio irregulariter intertexto consistente, hoc subhymenium circum spatia interlamellaria parallele sequente; sporis globosis, spinulis altis (0.8–1.0 $\mu$) cylindricis obscure ferruginosis obsitis, maiusculis, poro germinativo et callo destitutis; pigmento cuticulae pilei membranali; cuticula palisadam trichodermialem efformante; hyphis omnibus fibuligeris; mycelio albido, sparso, psammigeno; velo apparenter nullo. Species unica, **N. echinosporus** Sing. spec. nov., characteribus generis gaudet.

*N. echinosporus* has the characters of the genus (see above). The pileus is strongly depressed in the center; it has the size of *Deconica crobula* (or is slightly larger) for which it was mistaken by Rick, but is more fleshy and opaque; its color corresponds to "Spruce yellow" of Maerz & Paul. The terminal members of the trichodermium palisade are broadly clavate, 17–42 × 7–17 $\mu$. The lamellae are now a dull rusty color, deeply decurrent, occasionally with anastomosing veins, and often anastomosing at the stipe; the latter is, at least seemingly, evelate, rather thin but not cartilaginous or particularly fragile; the sterile layer of the lamellae consists of

[1] J. Rick, a pioneer-collector of South Brazilian fungi, died recently.

a subhymenium (small cylindric and isodiametric cells immediately underneath the basidia, well developed but not very sharply separated from the hymenopodium), a hymenopodium which is a broad layer of interlaced hyphae, only occasionally some hyphae running subparallel with each other (this layer accompanies the subhymenium all around the bottom of the interlamellar spaces as in the Boletaceae), and the mediostratum which lacks a lateral stratum, at least in the stage seen in the available specimens; the mediostratum proper consists of somewhat wavy, strictly axillary and loosely arranged hyphae; the spores are evidently some sort of brown when seen in mass but a good spore print was not preserved; under the microscope, they are brown-spinose (spines cylindric, 0.8–1.0 $\mu$ long), with a moderately thick wall, without a germ pore or callus, with a moderately large, central oil droplet, and 8.5–10 $\mu$ in diameter; the young, immature spores are smooth, then they become punctate, and finally echinate; the lower part of the spines may perforate part of the wall but this cannot be seen clearly with the methods employed; basidia 2- to 4-spored basidia (the 2-spored basidia sparse to as numerous as the 4-spored ones), 30–48 × 8.5–10 $\mu$; cystidia were not seen, but there are what may be called cystidioles on the sides of the lamellae, and especially at the edge, versiform, sometimes (on edge) consisting of several isodiametric cells, without contents, 18–50 × 6.8–8.2 $\mu$. The specimens were collected on sandy soil near Couto, Brazil, in 1936.

## 2. THE GENUS MACROMETRULA DONK & SING.

Massee described a species, obviously introduced to England, as *Agaricus rubriceps* Mass., and lists it in the Friesian subgenus *Chitonia*. The type of this species is preserved at Kew. The author wants to express his gratitude to the Director of the Kew Gardens, England, for loan of this material. Before, however, a systematic attempt had been made on the part of the author to determine into which genus this species actually belongs, Dr. M. A. Donk told the author that he considered Massee's species as the type of a new genus. It was then agreed to propose this genus —if new—under the name *Macrometrula* Donk & Sing. This new genus is—as shown by an extensive anatomical study of the

type by Singer—extremely close to *Psathyrella,* yet still well enough separated by one very striking character, the large, cup-shaped-saccate volva, and by some less important characters. The author has, indeed, hesitated for a while, waiting for more information on the genus *Psathyrella* that might provide a possibility to find transitional forms within the latter genus. The author wants to express his indebtedness to Dr. Alexander H. Smith, the American specialist of *Psathyrella* and related genera, for an interesting discussion on the subject of *Macrometrula* and its relationship with *Psathyrella.* It appears that *Macrometrula* is well enough separated from all groups of *Psathyrella* to justify a generic distinction of the former genus which is characterized by the following diagnosis:

**Macrometrula** Donk & Sing. gen. nov.

Habitu genus *Volvariellam* (*Volvariam*) in mentem revocante, sed lamellis adnexis; epicute pilei cellulari *Psathyrellarum* modo, ex elementis isodiametricis dense agglutinatis consistente, subbrunneolis KOH ope in strato inferiore cuticulae; basidiis magnitudine et forma ea *Psathyrellarum* revocantibus, tetrasporis; cystidiis sursum ventricosis et attenuatis vel subcapitatis ad apicem (typus *"Hygrophilum"*); sporis minutis, poro germinativo lato applanato instructis, duplice et crasse tunicatis, levibus, endosporio hyalino, pallide umbrinis in KOH, fortiter discoloratis ad ardesiacum $H_2SO_4$ concentrati ope; tramate hymenophorali pallide brunneolo, subregulari, cum sporis non-amyloideo; subhymenio densissimo, ex elementis minutissimis efformato; stipite centrali, cavo, exannulato, e volvà bene evoluta, basali, firma, membranacea, saccata oriente; carne alba, ex hyphis fibuligeris consistente. Ad terram in calidariis. Typus generis: **M. rubriceps** (Cooke & Mass.) Donk & Sing. comb. nov. (*Agaricus rubriceps* Cooke & Mass.).

FARLOW HERBARIUM,
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CAMBRIDGE, MASS.

# NOTES AND BRIEF ARTICLES

## Book Review

Smith, Alexander H., *North American Species of Mycena,* pp. i–xviii + 521. *pl. 1–99. text figs. 1–56.* University of Michigan Studies, Scientific Series, Vol. XVII. University of Michigan Press, Ann Arbor. 1947.

It is a pleasure to read a taxonomic work as well written as this comprehensive treatise on *Mycena.* The reader at once realizes that this is not merely another book on a genus of the fungi, but it represents Smith's own personal contacts with most of the 237 accepted species presented.

This work on *Mycena* has followed to a wise degree the modern trend toward natural divisions on morphological characters as opposed to artificial groupings which have been especially the vogue in previous classifications within the Agaricaceae. Smith says, "Closeness of relationship, which is what we are dealing with (within genera), is based on degree of similarity in characters accepted as fundamental. Since the taxonomist is working almost entirely from circumstantial evidence, there is not much to be gained by insisting that a classification be based on relationship when the point has been reached where affinities are difficult to ascertain and must be determined on secondary characters. Consequently, in my estimation, the most satisfactory arrangement of species will always involve some compromise between artificiality and naturalness."

In the segregation of genera somewhat similar to *Mycena* emphasis has been rightly placed on relationships and a broader rather than a narrower concept than that of Fries has been adopted. "It is evident that in this genus speciation has proceeded largely on microscopic characters, such as those of spores and cystidia, and because of this, I have not seen fit to use these at the generic level. By adopting a broad concept, the relationships of the species, particularly those which formerly were regarded as borderline species between *Mycena* and *Collybia* and *Omphalia,* could be more satisfactorily expressed. At the same time, *Mycena,* as a concept, is

maintained for a group of fungi so similar in general appearance that anyone with a small amount of experience can nearly always recognize it at sight in the field. To me, this is one of the criteria of a truly natural genus."

This comprehensive treatment of *Mycena* in North America satisfies a long-felt need by those who have in any measure attempted its study. Atkinson and Kauffman had started monographs of the genus, utilizing microscopic characters to delimit species, and Smith has expressed warm appreciation of the stimulus Kauffman personally gave through frequent discussions and Kauffman's wealth of knowledge of the genus gained by years of experience.

A concise but accurate history of *Mycena* in North America is included, giving recognition to the contributions of Schweinitz (1822–1834), Curtis (1867), Peck (1872), Harkness and Moore (1881), Murrill (1916), Kauffman (1918), and Beardsley and Coker (1924).

For the most part, American mycologists have saved type specimens of new species, making possible studies of microscopic details, and thus avoiding "the confusion of concepts that has characterized European agaricology since the time of Fries. However, since many American students did not pay close attention to microscopic details, numerous misidentifications have appeared in our literature, and a multiplication of names for some species has resulted."

Smith's description of *Mycena* delimits the genus quite precisely, as Kühner has done. "Although the arrangement presented here differs in some particulars from that of Kühner, it is in close agreement with his." Kühner recognized 143 species of *Mycena* in Europe whereas Smith accounts for 218 from the United States and Canada and 19 from tropical North America. The author regrets that due to the war, he was unable to make a comparative study of collections of *Mycena* in European herbaria.

The genus is divided into the four subgenera, Pseudomycena, Eumycena, Glutinipes, and Mycenella. These are subdivided into sections (and subsections in Eumycena) which are often again divided into stirpes. Pseudomycena includes two sections containing six species, Eumycena, 11 sections and numerous subsections containing 185 species, Glutinipes, four sections containing 21

species, and Mycenella has two sections containing six species. The section typical of Eumycena naturally forms the hub about which the other sections and subgenera are grouped, and contains the type species, *M. galericulata* (Fr.) S. F. Gray. The keys to the subgenera and sections are for the most part based both on macroscopic and microscopic morphological characters.

The author devotes a chapter of more than 20 very interesting pages to an evaluation of the various diagnostic characters used by him in speciation. Consideration is given to seasonal occurrence, habit and habitat, size and stature of fructifications, color, macroscopic characters of the surface of both pileus and stipe, lamellae, flesh, latex, macroscopic characters of dried specimens, and such microscopic characters as spores, basidia, cystidia, structure of gills and pileus, iodine reaction of the flesh, and viscidity of the stipe.

The author directs our attention to Kühner's [1] excellent and more extensive account of the anatomical details of the various types of carpophores grouped in *Mycena,* since the present work considers only characters emphasized by Smith in his arrangement and description of species.

There is included a short chapter on the technique of (1) collecting and drying specimens, (2) preparation and study of fresh and dried material, and (3) chemical tests.

The following 29 new species and seven new varieties are described: *Mycena albicolor, M. albissima, M. arenaria, M. Brownii, M. cayugaensis, M. cheboyganensis, M. cineraria, M. cylindrospora, M. filiformis, M. fuliginella, M. Gaultheri, M. griseoviridis, M. incarnatifolia, M. kalalochensis, M. Kauffmaniana, M. Kuehneriana, M. litoralis, M. paucilamellata, M. pseudoclavicularis, M. pseudogrisella, M. pseudoinclinata, M. Rickeni, M. setulosa, M. subcana, M. subconcolor, M. subfusca, M. tenuiceps, M. thujina,* and *M. umbrina; M. alnicola* var. *odora, M. epipterygia* var. *lignicola, M. flavoalba* var. *microspora, M. griseoviridis* var. *cascadensis, M. olida* var. *americana,* and *plumbea* var. *robusta.* There are three species newly named, *i.e., Mycena Atkinsoniana* (*M. fagicola* A. H. Smith, not *M. fagicola* Grog.), *M. rubrotincta* (*M. tenuicula*

[1] Kühner, Robert. Le Genre Mycena (Fries). Encyc. Myc. **10**: 1-710. 1938.

Murr., not *M. tenuicula* Karsten), and *M. subvestita* (*Omphalia vestita*, not *M. vestita* Velenovsky).

Among the excluded species is the new combination *Hygrophorus acutoconicus* (Clements) Smith (Syn. *Mycena acuto-conica* Clements), and the following 16 new combinations resulted from transfers from *Omphalia* or *Omphalopsis: Mycena albidula* (Pk.), *M. bisphaerigera* (Lange), *M. delicatella* (Pk.), *M. lilacifolia* (Pk.), *M. McMurphyi* (Murr.), *M. misera* (Fr.), *M. pallida* (Murr.), *M. papillata* (Pk.), *M. pseudogrisea* (Murr.), *M. pusillissima* (Pk.), *M. semivestipes* (Pk.), *M. serotina* (Pk.), *M. subimmaculata* (Murr.), *M. Swartzii* (Fr.), *M. translucentipes* (Murr.), and *M. turbinata* (Murr.).

The author is to be commended on the consistently good photographs well reproduced in 99 half-tone plates which illustrate in natural size 130 species and varieties.

There are also 56 full pages of text figures containing line drawings of the cystidia and spores of more than 200 species and varieties of *Mycena*.—S. M. ZELLER.

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## NOTICE OF FORAY

The Annual Foray will be held June 15–17 at the University of Michigan Biological Station on Douglas Lake, Cheboygan Co., Mich. The early date, necessary because of the September meetings of the A.A.A.S. and the Mycological Society, will give members an opportunity to see and collect a somewhat different flora from that found in early autumn. The region is rich in bogs as well as hardwood and coniferous associations. Dr. A. H. Smith, who has collected in the area in early summer for several seasons, states that such forms as *Underwoodia columnaris, Rhodotus subpalmatus, Otidella fulgens, Collybia tenuipes, Mitrula phalloides,* three species of *Crinipellis,* large numbers of smaller discomycetes, species of *Morchella* and *Helvella* and many species of *Mycena, Psathyrella* and *Inocybe* should be available. Laboratory facilities will be furnished by the station. Board and room will be approximately $3.00 a day per person. Communicate by June 10 with F. K. Sparrow, Botany Dept., University of Michigan, Ann Arbor, if you plan to attend. Give details of number of persons and length of stay.—F. K. SPARROW.